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GAMETOPHYTIC REGENERATION AS EXHIBITED BY MOSSES, AND CONDITIONS FOR THE GERMINATION OF CRYPTOGAM SPORES.

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A STUDY OF REGENERATION AS EXHIBITED BY MOSSES.

1. INTRODUCTION.

That the sexual generation of the Bryophytes is endowed with a remarkable power of regeneration is a well-known and oft-stated fact. The extent to which this is true for the liverworts has been shown by the investigations of *Vöchting*[1] "Über die Regeneration der Marchantieen" and of *Schostakowitsch*[2] "Über die Reproduktion und Regenerationserscheinungen bei den Lebermoosen". As far as the mosses are concerned, the generalizations have been based upon scattered and isolated observations by Schimper, Goebel and others, and not upon any detailed investigation. The present work has been carried out with the intention of showing to what extent these generalizations in regard to the vegetative reproduction from stem and leaf are true, and also to throw some light on the physiology of regeneration.

Before proceeding with the results of my own investigations, brief mention will be made of some of the observations previously recorded.

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[1] Jahrb. f. w. Bot. 16:367. 1885.

[2] Flora, Erg.-Bd. 350—384. 1894.

2. HISTORICAL.

The first record of the formation of protonema by the leaves is by *Kützing,*[1] for Bryum pseudotriquetrum. The leaves produced an abundant protonema growth and after a period of eight weeks, buds appeared.

Schimper[2] obtained a growth from the basal portion of detached leaves of Funaria hygrometrica, but no buds were produced. He also makes the very broad statement: "Chaque feuille et même chaque portion de feuille detachée de la plante-mere et placée dans les conditions convenables peut produire des filaments proembryonnaires, par la multiplication d'une ou de pleuiseurs de ses cellules parenchymatuses." *Goebel*[3] also mentions the ability of Funaria leaves to produce protonema, when they are detached and kept moist. *Limpricht*[4] states that almost every leaf can by proper culture be made to form secondary protonema. Also in the case of plants with brittle leaves as, Leucobryum glaucum, Barbula fragilis, Campylopus fragilis, and Barbula ruralis, one can find in nature on the detached leaves the begennings of protonema filaments.

It is to be noted that in all of the cases above mentioned, regeneration only occurred when the leaves were detached from the stem. That this is not necessary in all cases is shown by the observations of Goebel[5] on the leaves of several species. In Oncophorous glaucus a thick felt of tangled filaments appears on the fertile summits of the plants, which prevents their further growth and eventually gives rise to patches of young plants. The marginal cells of Buxbaumia aphylla leaves are able to produce protonema which will completely envelop the leaf. According to Limpricht,[6] the apex

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[1] Phycologia generalis p. 282. 1840.

[2] Recherches anatomique et morphologique sur les mousses, p. 19. 1848.

[3] Sitz.-Ber. d. mat.-phys. Classe d. k. bayr. Akad. d. Wiss. 26 : 463. 1896.

[4] and [6] Laubmoose von Deutschland 1 : 64.

[5] Outlines of Classification p. 173. 1887.

of the end bud in Leucobryum has been known to produce a protonema growth, and H. Schulze has observed a luxuriant growth of protonema filaments from the leaf apices of Hypnum giganteum.

Mention should be made here of the formation of brood bodies on different portions of the leaf, now apex, now costa, in various species of Orthotrichum, Ulota, Barbula, Grimmia, Syrrhopodon and Calymperes.[1] These brood-bodies are apparently formed in the young stages of the leaf and are homologous with protonema productions. They become detached from the leaf and under proper conditions grow out into protonema filaments, although in some cases growth may begin before detachment.

The formation of a protonema filament and the later production of a new plant has been observed from the calyptra of Conomitrium Julianum. According to Goebel[2] the formation was from the inner side, and according to Schimper,[3] from the outer surface. Limpricht[3] has also recorded the production of protonema by the detached calyptra of Phascum.

Limpricht[4] ascribes to all parts of the moss plant a very great power of regeneration since he says: "Alle Teile der Moospflanze besitzen die Fähigkeit, sekundäre Protonema zu erzeugen," and specifically in regard to the stem: "Auch jede Zelle der Stengeloberfläche ist fähig, einen Protonemafaden zu bilden." In a great majority of cases however an intervention of a rhizoid production occurs. The sessile or stalked brood-bodies of Pleuridium alternifolium originate from the stem. Bryum erythrocarpum[5] produces axillary brood-bodies, and Webera annotina and Ludwigii[6] produce axillary bulbils which detach themselves from the stem and grow

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[1] Goebel, Outlines of Classification, p. 172—73. 1887. Limpricht, Laubmoose, 1 : 64.

[2] l. c. p. 173.

[3] Limpricht, Laubmoose 1 : 65.

[4] l. c. p. 61 and 63.

[5] Schimper, Rech. anat. et morph. sur les mousses, p. 19. 1848.

[6] Schimper, l. c. p. 7.

without the intervention of any protonema. *Schulze*[1] records the production of bulbils by the stem of Hypnum aduncum, which detach themselves and grow in a similar way. The brood-bodies of Aulacomnium and of Tetraphis pellucida also originate from the stem. Mention should also be made here of the work of Müller Turgau[2] on the production of "Zweig-vorkeime".

Not only the gametophyte but also various parts of the sporophyte are able to produce protonema. This has been observed by Stahl[3] from the capsules and setae of Ceratodon purpureus, and by Pringsheim[4] for Hypnum serpens and cupressiforme, and Bryum caespitosum, all in artificial cultures, and by Brizi,[5] in nature for Funaria hygrometrica. According to Brizi, some of the setae of Funaria which had come into contract with the earth produced an abundant growth of protonema with numerous buds.

3. METHOD.

In course of the experiments described below three different methods were used. The leaves and stems to be used as cultures were carefully washed in sterilized water in order torender them as free as possible from bacteria and fungi, and then placed either in Petri-dishes upon several thicknesses of filter paper which had been saturated with a nutritive solution, or upon pieces of flower pots placed in crystalizing dishes. In the third method the leaves were placed upon soil in either Petri or crystalizing dishes. The filter paper was carefully sterilized in boiling water and then placed in the Petri-dishes which had been previously sterilized in the dry-oven. The pieces of flower pot weres first boiled and then sterilized together with the crystalizing dishes in the dry-oven.

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[1] Bot. Centralblatt 31 : 382—384. 1887.
[2] Arb. d. Bot. Inst. Würzb. 1 : 475—499. 1874.
[3] Bot. Zeitung 34 : 690. 1876.
[4] Jahrb. f. wiss. Bot. 11 : 1—46.
[5] Annuar Instituo botan. Roma 5 : 53—57. 1892.

The dishes containing the soil were also sterilized in the same way. All of the cultures were supplied with a $^1/_4$ pro mille normal nutritive solution, and were kept at a temperature varying between 19—21 ° C.

4. EXPERIMENTAL.

In course of my investigations the following species were used:

1. Mnium rostratum Schwagr.
2. Funaria hygrometrica Hedw.
3. Bryum capillare Hedw.
4. Bryum argenteum Linn.
5. Barbula muralis Timm.
6. Atrichum undulatum P. Beauv.
7. Polytrichum commune Linn.
8. Brachythecium rutabulum Nob. and variety.
9. Leptobryum pyriforme (L.) Schimper.
10. Phascum cuspidatum Schreb.
11. Ceratodon purpureus Brid.
12. Fissidens bryoides Hedw.

In addition to these, cultures of Plagiochila asplenoides and Lophocolea bidentata were made for comparison with those of Schostakowitsch.

MNIUM ROSTRATUM.

Mnium on account of the size of its leaves and the consequent ease of manipulation presents a very favorable specimen for experimentation. In its power and manner of regeneration it stands alone among all of the species investigated. At first two cultures were made for exposure to light; the leaves were carefully stripped from the stems and in one case placed with the dorsal surfare upper most, in the other with the central surface upper most. These cultures were placed

upon a table in the middle of the laboratory. Two similar preparations, were made and enclosed in a dark chamber.

After an interval of a week the first appearance of rhizoids from the leaves was noted. An examination of the specimens grown in the light showed that the rhizoids proceeded almost exclusively from the contact surface, and in general from the periphery of the leaf, although they were not entirely absent from the middle and costal region. An examination of the cultures in the dark showed nearly the same manner of growth except that a considerably larger number of rhizoids originated from the side upper most, the proportion being about 1 to 10. The rhizoids from the very first, in both light and dark were devoid of chlorophyll and the cell-walls were distinctly brown. As growth proceeded, those in the light developed an abundance of chlorophyll bodies and showed in nearly every case oblique cross-walls. In the course of two weeks the rhizoids in the light had branched considerably, while in the cultures in the dark they rarely branched, and the cells were more elongated. At the end of three weeks, the first appearance of buds was noted; and in cultures in brighter light in the window after a lapse of two weeks. The buds originated exclusively from the illuminated side and directly from a leaf cell without the intervention of any protonema. The buds generally made their appearance near the periphery of the leaf and the cell from which the bud originated had previously given rise to a rhizoid from the contact side. This is shown in cross sections of the leaf in Fig. 2 and 3. The mother cell of the bud first produces a protuberance which becomes divided very soon by an oblique wall, and the insertion of the successive walls then follows in rapid order. Buds may occasionally originate as side branches of the rhizoids from either surface, although this is rare in the normal development. At the end of six weeks, the specimens in the dark showed no sign of buds, and the long unbranched rhizoids had attained a length of about one centimeter. The peculiar method of regeneration shown in these experimentes is

especially noteworthy since Goebel [1] states that the vegetative reproduction of mosses has this peculiarity, that the formation of new leafy shoots is always preceded by the production of a protonema.

From the above experiments it is demonstrated that there is no inherent tendency to the production of rhizoids or buds from a particular side of the leaf; that buds are not produced in darkness, either because the photosyntactic processes cannot be active or because light in itself is necessary. The greater production of rhizoids from the free side of the leaf in the dark would indicate that illumination exercised a retarding influence upon their production. The growth of the rhizoids from the contact surface of the leaf may be due either to contact or gravity, or both.

In order to determine the part which contact and gravity play in the direction of rhizoid growth, the following experiments were carried out. Leaves were placed on filter paper and grown in the dark in an inverted position, and in these cultures the same as in the ordinary position, the leaves produced rhizoids mostly from the contact surface. In order to render the supply of moisture of both surfaces as nearly equal as possible, the leaves were grown in a saturated atmosphere. Other leaves grown in both light and dark between two sheets of filter paper showed a production of rhizoids about equally from both surfaces. Again, leaves which were grown in a vertical position produced rhizoids radially in all directions. These experiments then show that the rhizoids are not influenced as to their point of origin by gravity but more by contact. Leaves were also grown in soil with about the same result except that a greater number of rhizoids originated from the surface of the leaf nearest the air. The formation of buds upon the leaf in the ordinary manner was naturally prevented and when the rhizoids reached the surface of the soil and were

[1] Outlines of Classification, p. 170. 1887.

exposed to light, they gave rise to an abundance of protonema like branches and numerous buds.

A culture of leaves with long, sparcely branched rhizoids which had been grown in the dark was removed to the light and allowed to undergo further development. When examined a week later the rhizoids had produced in the apical region an abundance of branches part of which were still rhizoid in character. A large number of the branches were howerer distinctly protonema in nature, the cell-walls colorless, the cross walls perpendicular, the cells short and filled with an abundance of oval chlorophyll bodies. The rhizoids also contained chlorophyll bodies but there were fewer in number and of an elongated lenticular form. An enormous number of buds was also formed and in either of two ways: either as a direct modification of a side branch from a rhizoid cell, or as a side branch from one of the lateral protonema branches. This is plainly illustrated in Fig. 6 and 7. Occasionally a bud was formed later near the leaf, but the great majority made their appearance towards the distal exteremity of the rhizoids.

A question which now presented itself was: Is the continued exposure to light necessary to call forth the production of buds? In order to determine whether buds would be produced by light induction, leaves were grown in bright light for nearly two weeks and then carefully examined to see that no buds had been formed. They were then placed in the dark chamber and after five days the formation of buds was observed. The number was much less than from those leaves in the light, and on account of a lack of food material only a limited growth occurred. Whether this light induction is due to physical or chemical changes in substances already present in the leaf, or to the accumulated products of photosyntax, can not be stated with certainty, but the experiment which follows would indicate that the products of photosyntax are not necessary to call forth the production of a leafy shoot.

In order to determine whether the products of photo-

syntax as obtained from the use of the free CO_2 of the atmosphere are necessary to call forth bud production, a culture of leaves was made in CO_2 free air in au apparatus similar to that figured by Pfeffer.[1] At the end of three weeks the leaves showed a very abundant production of buds. It has long been known that plants are able to use the CO_2 of respiration as material for photosyntax. Since this is so, the above experiment does not prove conclusively that light is necessary to effect physical or chemical changes in material already present, for on account of the size of the Mnium leaf, the CO_2 product from the destructive metabolism would be considerable and a small amount of carbohydrate food might be formed. Later experiments with other species tend to show that it is the accessible supply of plastic material upon which the production of buds is dependent, and not upon physical or chemical changes in the material already at hand.

Experiments with leaves in colored light by the use of the double-walled bell-glasses filled with the solutions of potassium bichromate, and ammoniated copper oxide, showed the production of buds as well in the strongly refrangible rays as in the less refrangible. The photosyntax would be greatly suppressed in the leaves exposed to the blue end of the spectrum, and thus this result points to a chemical or physical change in material already at hand. Since Klebs[2] has pointed out a difference in the relation of spore protonema and leaf protonema to light in a specific case, we might reasonably expect to find a difference in the leaf productions from different species. Another point which may be noted in the case of the cultures in the rays of different refrangibility is that in both the strongly refrangible and less refrangible rays, the leaves produced a much greater number of rhizoids from the surface uppermost. This would tend to corroborate the statement already advanced that

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[1] Pflanzenphysiologie 1 : 191. 1881.

[2] Biologisches Centralblatt 13 : 646—648. 1893.

light retards the production of rhizoids, since here each culture was only subjected to half the rays of the spectrum.

In all of the cultures the buds only originated from the illuminated side of the leaf and the question naturally suggests itself: Is this due to illumination or to the negative geotropism of the moss shoot? In order to determine this, a series of leaves was illuminated from below by a mirror, so that light and gravity would be acting in the same direction. After the usual length of time buds made their appearance and that only from the illuminated surface. Bastit [1] has shown that the moss-plant is distinctly negatively geotropic, but that with illumination from below, the shoots grow towards the light, the influence of gravity being overcome by that of light. This I have been able to substantiate in the case of plants grown from the leaves. Another series of experiments was carried out with leaves illuminated from both surfaces. In order to effect this, the leaves were placed in a Petri-dish and irrigated by means of narrow strips of filter paper alternating with rows of the leaves. The dish was placed upon a ring-stand and illuminated from below by a mirror. In this experiment I found that the buds originated from both surfaces, thus showing the dependence upon illumination. In another series of cultures the leaves were placed in a vertical position in the soil and in such a manner that the leaf surfaces were parallel to the incident rays of light. These as well as the previous experiments showed the production of buds from both surfaces.

In the case of whole leaves the buds appeared only near the periphery and within the leaf margin, the cells of the border never producing any growth. The cutting of the leaves transversely did not alter their power of regeneration, both rhizoids and buds being produced in as great abundance as in the whole leaves. In order to show

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[1] Rev. Génér. de Botanique 3: 406—411. 1891.

whether it was possible for the cells from the costal region to give rise to buds, the lateral halves were split away from the costa, and both portions cultivated. The result was that buds appeared from the costal region as from the lateral halves, showing that in the whole leaf the power to produce buds was only suppressed. Again with reference to the power of young and old or fully mature leaves to regenerate. Series of leaves from the mature to the very smallest that could be dissected from the end bud were subjected to culture, with the result that the leaves from ordinary size to about half way through the series produced buds and rhizoids in abundance. Those from this point on to the very minute leaves produced only rhizoids, and these mostly from the region of the costa. It was evident that the plastic material was not present in sufficient abundance to produce a further development, or that being an embryonie organ, the young leaf used its available supply of food material towards the growth of its own cells.

So far as I have observed, the leaves of Mnium in nature never give rise to rhizoids when still in connection with the stem. In order to afford experimental proof of this, whole plants were subjected to exactly the same conditions as the detached leaves, but no rhizoid production resulted. Again, it might be thought that the formation of rhizoids and buds was called forth by the injury to the leaf. That the cutting of the leaf is not effective in the production was shown by those experiments in which the leaves were cut and still left in connection with the stem, even in these leaves no new growth resulting. Another series of experiments was made in which the costa was cut near the base of the leaf while the lateral halves were still left in connection with the stem, with the idea that the severing of the costa might cut off the path for the transport of food material. No rhizoid growth was called forth, and hence the previous experiments show that nothing but the complete separation of the leaves from the the stem is able to call forth the power of the leaf to regenerate. When

the leaf is still in connection with the stem, the plastic material can be transported to other younger and growing parts; in the detached leaf on the other hand the escape is cut off, and thus may favor the production of rhizoids and buds. The simple cutting of the leaf in itself seems to be however the important factor, that is, the complete separation of the leaf from the stem affords the stimulus for growth, which is then applied to the production of rhizoids and new leafy shoots.

When the stems of Mnium are stripped of leaves and kept in conditions favorable for growth, they will produce new shoots which originate as axillary branches. As is often noticed in nature, the stems produce an abundance of rhizoids and these in greater abundance from the region of the stem which has given rise to a shoot. In no case however was a production of protonema direct from the stem to be observed, and the rhizoids grew for months without giving rise to any protonema branches. The production of new shoots from the stems occurred as well in the dark as in the light; in the dark however the new shoots produced smaller leaves, and were more slender and elongated. The shoots used for experimentation were laid horizontal, and the lateral shoots grew erect, both in the dark and in the light, thus showing a well marked negative geotropism. The production of the new shoots was not called forth by the defoliation, but only accelerated thereby, since whole plants subjected to the same conditions produced new shoots as lateral branches, according to the manner of branching in nature. The stems also showed quite a distinct tendency to the production of shoots from the region of the morphological apex. Defoliated stems were grown in a vertical position in a moist chamber, part with the morphological apex uppermost, part with it directed downwards. The result was that in the majority of cases the new shoot appeared a short distance below the apical end. In some cases the stems gave rise to to several shoots, and some of these were often well removed towards the basal end. The

new shoots produced from the stem as well as those produced from the leaves were distinctly positively heliotropic. By reversing the leaf cultures from time to time after they had reached the length of a few millimeters, the stem was made to assume a zig-zag form due to the heliotropic curvatures.

It may be noted here that the leaves generally formed 10—15 buds, but only two or three of these continued their development to any considerable size. It has been already noted, that leaves in which the bud production was prevented by darkness, produced protonema from the apical portion of the rhizoids when subjected to light. In case however the normal production of buds direct from the leaf was allowed to be carried out, the rhizoids did not produce any protonema branches, and ceased growth soon after the new plants had been formed.

FUNARIA HYGROMETRICA.

The production of protonema by the leaves of Funaria has already been mentioned in the references to the researches of Schimper, Goebel, and Klebs. Goebel states that he obtained protonema in great abundance from Funaria leaves, but my experiments do not show the leaves to be endowed with a very great power of regeneration. The plants used were taken from the green-house and were apparently in vigorous condition. Cultures of leaves were made in the same way as for the Mnium leaves, and placed in both light and dark. On an average of about one out of every six leaves showed signs of protonema. In all the cases noted in the first series of experiments, the growth was entirely from the cells of the base and only from those which had been directly attached to the stem. The cultures which were grown in the dark showed growths of a decided protonema nature, the cell walls colorless, the cross walls generally a little inclined and cells filled with bodies irregular in outline, and without any green color. The filaments

remained long and almost unbranched, and reached a length of about 1 cm. Several cells of a filament grown in the dark are shown in Fig. 9 for comparison with those grown under normal illumination.

In one or two cases the leaves produced structures which were more rhizoid in nature, and these in the cultures both in the light and in darkness. In all of the cultures no buds were produced in the dark, while under normal illumination they appeared after ten days to two weeks. The protonema very soon after its origin from the leaf, often gave rise to a bud as a lateral branch, and numerous cases were observed in which this bud formation occurred from the second protonema cell. This is illustrated in Fig. 8.

In two cases out of all the experiments which I carried out, I found a protonema production from other than the basal cells, so it would seem that the cells of the basal portion of the leaf are more inclined, to produce protonema than those from other parts. In the preparation of the cultures the leaves were stripped from the stem with a pair of forceps, and occasionally portions of the stem were torn away with them. A very abundant production of protonema occurred from these portions of the stem. In order to show whether the power of regeneration was localized more in the basal cells of the leaf, a series of cultures was made in which the entire basal portion of the leaves was cut away. These cultures were kept for six weeks and at the end of that time no formation of protonema had occurred. That the power of protonema production is not confined entirely to the basal cells is shown by the two cases already mentioned where protonema were produced from the region of the tip. Hence, the experiments only show that the leaf cells adjacent to the stem produce protonema more readily.

Whole plants brought under exactly the same conditions as the detached leaves did not produce any protonema from the leaves, and again plants with the leaves cut away at the tip showed no signs of protonema production. From the experiments it must be concluded that the complete separation

of the leaves from the stem is necessary in order to call forth the formation of protonema.

The experiments with the leaves which had portions of the stem torn away with them showed the stem cells to have a remarkable power of protonema production. A series of cultures was made in which the leaves were entirely stripped from the stems and the stems cultivated in both light and dark. The stems produced new shoots as lateral branches with remarkable rapidity. After a lapse of only three days the new shoots had reached a length of nearly two millimeters. No distinct tendency to the appearance of the new shoots from the region of the morphological apex of the old shoot could be detected. Generally, however, a shoot was formed just back of the apex, but in the majority of cases they were produced at other points along the stem, and even from the very base. Occurring at the same time with the production of new shoots was an abundant growth of protonema from the stem for its entire length. The regeneration by new shoots was always in the way of axillary branches, in a manner similar to that which often occurs in nature. The protonema were not however confined to the leaf axils but grew as well from cells removed from the axillary regions. In the cultures in the light, the protonema originated generally from the side of the stem which was uppermost, while rhizoids were produced from the contact side and in greater abundance from the region of the stem which had formed a new shoot. This is shown in Fig. 12. The cultures in the dark showed very rarely a protonema production, and in neither light nor dark was any bud formation noted from the stem protonema. In several cases where the receptacles with the perichaetial leaves were placed in culture, an abundant protonema production was noted from the end cells of the receptacle. A dissection showed these protonema to originate from the cells lying between the bases of the antheridia archegonia, and paraphyses, and also from the basal cells of the paraphyses as shown in Figs. 10, 11. All attempts to obtain protonema from the paraphyses when separated from

the stem were without effect. The material for growth was evidently drawn from the stem, and when this supply was cut off, the cells were not capable of independent growth.

In order to determine whether the production of new shoots and protonema was called forth by defoliation or not, whole plants were placed in exactly the same conditions as the defoliated stems. Regeneration by means of new shoots occurred, but not in the abundance that was noted in the defoliated stems, while no production of protonema occurred and only occasionally rhizoids. The production of protonema was then called forth by defoliation; the formation of new shoots was only accelerated by the defoliation.

A fact which must be of importance to Funaria was shown in the experiments in which whole plants and defoliated stems were placed under earth at a depth of 3 mm. The stems in both cases formed lateral branches which grew erect from the stems which had been burried in a horizontal position. After a lapse of two weeks these new shoots first made their appearance above the soil. Considering the habitat of Funaria the power of regeneration in this manner is of considerable importance in nature, since the plants often become covered with soil and would otherwise perish.

The new shoots from the stem as grown in dark, were about twice as long as in the light cultures, and the leaves were much reduced in size. The cultures in the light showed the new shoots to be strongly positively heliotropic. In the dark the new shoots grew erect from the prostrate stems. Stems were placed in a Petri-dish in the ordinary horizontal position, and the dish then inverted. The new shoots curved around so as to grow upwards, showing them to be distinctly negatively geotropic.

BRYUM CAPILLARE.

The leaves of B. capillare show a very remarkable power of regeneration. Cultures of the leaves were made the same as for Mninum, and part placed in the light and part in the

dark chamber. At the end of a week the majority of the leaves used had produced new growtbs, and these mostly from the basal portion of the leaf. The first growth from the leaf cells was of neither a pronounced rhizoid or protonema nature; the walls were colorless, the cross walls occasionally perpendicular but more generally slightly oblique. With exposure to light the filaments tended to a growth of a more decided protonema nature, the cross walls were predominately perpendicular in the abundant lateral branches, and quite often in the main axes also, and the cells soon developed an abundant chlorophyll content. With time the walls of the main axes turned brown, and the chlorophyll content disappeared, so that eventually the main axes, even though exposed to the light, came to resemble rhizoids. With the continued exposure to darkness the filaments soon became brown; no chorophyll was formed, and the lateral branching was very generally suppressed. In the cultures in the dark no buds were formed, while in the light cultures the first buds were noticed at the end of seven days, with the more abundant production as growth continued. The buds originated as side branches of the main axis soon after the filament had grown from the leaf cell. In the further growth the buds appeared at different points along the main axis and were homologous with the lateral protonema branches. The lateral branches might also in their turn give rise to buds as lateral branches, and after six weeks an enormous number of new plants was produced in this way.

The protonema production occurred generally from the cells of the the leaf base, either from the marginal cells or from those of the lacerated base, more general than from the cells in the interior of the leaf. Although protonema originated from the cells removed from the periphery, no distinct tendency to production from a certain side of the leaf was noted. Part of the protonema would originate from the contact surface and part from the free surface, sometimes more from the contact surface, sometimes more from the opposed surface, so that no constant effect of contact was

demonstrated. Leaves which had remained in the dark for two weeks had produced long, sparcely branched rhizoids without any signs of buds. They were then placed in the light and after the lapse of ten days abundant protonema branches were produced from the distal portions of the rhizoids, and also an abundance of buds, thus showing that light was necessary for the formation of buds. Luxuriantly growing protonema without any buds were placed in the dark and allowed to remain for two weeks. The specimens were grown upon pieces of flower pots and at the end of the two weeks no buds had been formed, although the protonema from its previous exposure to light must have contained a considerable supply of plastic material, which was used in continued growth rather than in the formation of leafy shoots. No structures at all resembling rhizoids were produced and at the end of the experiment the protonema filaments were beginning to die from lack of food material. From these results it will be seen that in the case of B. capillare a continued exposure to light is necessary for the production of buds.

In order to determine whether the cells removed from the basal region of the leaf were able to produce protonema as readily as those of the base, a series of cultures was made in which the leaves were cut transversely through the middle, and both basal and apical portions retained in culture. The basal half of the leaf produced protonema from both the proximal and distal ends, but only rarely from the cells occupying the interior. The apical half of the leaf also produced protonema from the cells next the cut base. (Figs. 17, 18, 19.) Another series of cultures was made in which the leaves were cut lengthwise, and these showed protonema production from the base and also from the cut margins. These experiments then show that almost any cell of the leaf may grow out into a protonema, but that in the cells with one side next the margin, the tendency to form protonema is greater than in those cells which are surounded on all four sides by others.

The experiments with whole plants placed under like conditions as the separated leaves, showed no protonema production whatever from the leaves, and when the tips of the leaves in whole plants were cut away, even then, the leaves formed no protonema. Thus, nothing more or less than a complete separation of the leaves from the stem would suffice to call forth the power of the leaf cells to grow out into protonema filaments.

Experiments with leaves grown in blue and red light brought a different result than was found in the case of the Mnium leaves. The leaves in the red light produced buds, apparently with as great readiness as in normal illumination, while in the blue light no buds whatever were formed. When we reflect that it is only in the red light that photosyntax takes place to any extent, the importance of this process as furnishing material for the formation of buds is at once made evident. That the products of photosyntax are necessary for the formation of buds is shown by the fact that leaves grown in a CO_2 free chamber also produced no buds. The results of there experiments with Bryum leaves accord with those of Schostakowitsch[1] for the foliose Jungermannieae, and those with Mnium agree partially with the results for thalloid liverworts. Experiments with Marchantia and other thalloid liverworts showed that regeneration occurred in the dark as well as in the light. I have also confirmed these results in the case of Marchantia but in the case of Lophocolea bidentata my results were different than obtained by Schostakowitsch for the same species. I found that the detached leaves produced buds from the marginal cells of the leaf, and that this production occurs quite abundantly in the dark as well as when the leaves are exposed to light. This result is more in accordance with the observation of Klebs.[2] According to Klebs the leaves of Lophocolea bidentata produced buds in a weak light at an intensity which was not sufficient to produce the germ disk in the case of spore-protonema. Mention may be

[1] Flora, Ergänzungsband: 380—384. 1894.

[2] Biol. Centralblatt 13: 649. 1893.

made here of the cultures of Plagiochila asplenoides leaves. Green-house specimens showing every appearance of vigor were used and the cultures were kept for over two months, but although the leaves remained green and vigorous, no sign of any bud or rhizoid production was observed. This was one of the species which Schostakowitsch grew successfully, and it is apparent from there results that there are conditions of the plant, when althougly apparenth vigorous, the power of regeneration may be suppressed.

The defoliated stems of Bryum produced some protonema direct from the region of the leaf axil, but in the case of specimens grown in the dark no distinct protonema growths were noted. The abundance of production was much less than in the case of Funaria hygrometrica. The paraphyses here also were able to grow out into rhizo-protonema, by the continued growth of the distal cell. This occurred however only when they remained in connection with the stem, all attempts at cultivating the detached paraphyses being to no avail. The stems produced rhizoids quite abundantly, both in ligth and darkness, and the production was not confined to any particular portion of the stem. From the rhizoids an abundance of buds was formed as lateral branches, and in a light intensity which was not sufficient to produce vigorous protonema. New shoots were produced by the stems as lateral branches the same as in Funaria. These appeared without any distinct localization of the point of origin, coming now from near the tip and now near the base of the stem. The production of protonema was due mostly to defoliation of he stem, since only in rare cases was a protonema production noted from the whole plants which were kept in the same conditions as the defoliated stems. Rhizoid production was quite abundant from the whole plants, but the growth in general was more abundant from the defoliated stems. The production of new shoots was not called forth by the defoliation of the stems, but was only accelerated thereby, since whole plants also formed lateral axillary branches, a mode of growth which is often

resorted to in nature, the new branches afterwards becoming separated from the parent plant. The whole and defoliated stems when burried under three millimeters of earth and kept moist also gave rise to lateral branches, which grew in the normal way, and by rapid growth soon appeared above the soil, the same as in Funaria. The importance of this power of regeneration in nature has already been emphasized in the case of Funaria.

The statements in regard to the elongated growth of the new shoots in the dark, with the development of reduced leaves, and the well-marked negative geotropism and positive heliotropism, hold good here as well as for Funaria.

BRYUM ARGENTEUM.

The manner of regeneration from the leaves of B. argenteum is so similar to that already described for B. capillare, that a detailed description will not be necessary. The whole leaves produced protonema from the basal portion, and the cut leaves from all of the cut edges. The character of the growth from the leaf cells was practically the same. The formation of buds occurred in abundance in the light cultures, but none in the dark. The formation of protonema due to the separation of the leaf from the stem, and not to the mere cutting.

An abundant protonema production occurred from the defoliated stems, the growth taking place from the region of the leaf axil. The protonema nature was generally suppresed in the dark cultures, only in a few cases long, unbranched, protonema like growths being noted. The protonema in the light produced buds in great abundance, and often as lateral branches of the first cell of the protonema filament. No buds whatever were formed in the dark. The protonema production was called forth by defoliation, since the whole plants only produced rhizoids, and not in the abundance which was noted in defoliated stems. As opposed to the other species studied, the defoliated stems did not produce new shoots as lateral branches, while the whole plants under

exactly the same conditions did. This is presumably explained by the small weak stem, which when robbed of its leaves is not able in itself to afford material for the growth of new shoots, in additions to what is used to produce the abundant growth of rhizoids and protonema.

BARBULA MURALIS.

The leaves of Barbula produce protonema with great readiness. Cultures of the detached leaves were made for both light and dark, and the best results were obtained from those upon pieces of flower-pot. After a lapse of about a week an abundant growth had appeared in the cultures in the dark as well as in the light. The first growth was colorless, with slightly oblique cross-walls, and no chlorophyll except what was derived from the leaf cell. Those which remained in the light for the entire period soon showed a very vigorous growth, with luxuriant branching and the absence of any bud formation. The walls of the main axes after a time turned brown and had more of a rhizoid nature. The side branches, although at times slender and tapering and now with oblique cross-walls, now with perpendicular walls, were decidedly protonema in character and possessed an abundant chlorophyll content. A thick net of interlacing protonema filaments was obtained from the culture in the light. At the end of ten weeks the network was several centimeters in extent, and not withstanding the fact that it had been exposed to the light in the laboratory window, no bud formation had resulted. The suppression of bud formation could not have been due to the lack of sufficient light, since as exposed in the window the illumination was quite intense. Up to this time the culture had produced no growths which I could call rhizoids. The cultures which remained in the dark produced only long, very sparcely branched filaments which in their further growth tended more to a rhizoid nature, with no chlorophyll, brown walls and always oblique cross-walls.

At the end of about eleven weeks the protonema had given rise to distinct rhizoid branches, and an abundance of buds had been formed. Soon after this the old protonema began to turn brown and die. During this period of growth, the extensive network of protonema filaments had not been entirely produced by the direct growth of the originally formed main axes, but a multiplication of the protonema had occurred. Certain side branches seemed to be specialized for this purpose, since the cells increased in size, developed a very abundant chlorophyll content, rounded themselves somewhat until they were about barrel-shaped, and then separated from the branches either singaly or several together. These separated cells then gave rise to new protonema. Goebel[1] mentions the pover of a protonema, species not known, to separate in this way when the culture was allowed to dry. In the case of Barbula however, the splitting away of the cells was not due to drying out, since the culture was supplied with nutritive solution for the entire period of growth.

The protonema originated only from the basal cells of the leaf, generally either from the very end cells or from those next the margin. The cells of the basal portion are much longer than those occupying the apical portion, and the question now presented itself as to whether the small cells of the apical half of the leaf were capable of growing out into protonema. In order to determine this the basal portions were cut away from a series of leaves, and both apical and basal portions retained in culture. The result was that no protonema were produced from the apical portions of the leaves, while the basal portions only produced protonema from the cells of the proximal end. The protonema growth was generally from cells occupying the periphery, but occasionally one originated from a cell a little removed from the margin. These experiments then show the power of regeneration to be confined to the larger cells of the leaf base.

[1] Sitz.-Ber. d. mat.-phys. Classe d. k. Bayer. Akad. d. Wiss. 36: 641. 1896.

In the material which was accessible to me, most of the stems were bearing young sporogones and had produced in their normal growth an abundance of rhizoids. The defoliated stems when placed in culture did not give rise to any new shoots and no appreciable production of rhizoids was to be noted.

ATRICHUM UNDULATUM.

So far as my knowledge goes, no moss-leaves with a structure similar to that of Atrichum leaves have been known to give rise to protonema. Hence the successful growth of protonema from these leaves is of the more interest. Four cultures of leaves were made: a series of leaves with the dorsal surface uppermost, and another series with the ventral surface uppermost, both to be placed in the light; two similar series were placed in the dark chamber. At the end of a month the first signs of protonema were observed and in the course of a week they had grown to a considerable lenght. An examination of all the cultures showed that the protonema in every case originated from the ventral surface of the leaf without regard to the position which it had occupied in the culture. And further, the examination of the whole leaves showed that the protonema originated from the cells lying at either side of the lamellae. The protonema production from this region was quite general throughout the entire length of the leaf.

In order to determine more closely the origin of the protonema cross sections of the leaf were made. The sections showed that the protonema originated from the large cells of the costal region lying at the base of the outer lamellae. (Fig. 32). The first growth from the leaf, although the cross-walls were predominately oblique, were decidedly protonema in character and remained so whether the specimens were grown in the light or dark. The branching was often aggregated in a manner altogether unique, as is shown in Fig. 30 which may be taken as a typical example. In other cases it was more as in ordinary protonema, but the only

difference between the protonema grown in the dark and those grown in the light, was that in the dark cultures the cells were generally more elongated and devoid of chlorophyll, and the branching less. The cell walls in both cases were colorless.

After about five weeks buds made their appearance, and always as modifications of lateral protonema branches. Contrary to what has been described for all of the preceding species the buds were formed in as great abundance in the dark cultures as in the light. In the dark the buds did not attain any considerable size on account of the lack of food material, rarely reaching a length of 1 mm. The production of buds in the dark is evidently explained from the nature of the leaf. The lamellae and the lateral portions of the leaf, since they give rise to no protonema are able to furnish considerable food material which can be applied to the growth of leafy shoots. These experiments show that in one case at least light is not necessary for developing and unfolding that slumbering "Anlage" of which Klebs[1] surmises the existence.

In order to determine if a correlation existed betiween the lateral halves of the leaf and the costal region in the production of protonema, the lateral halves of a series of leaves were separated from the costal regions, and both retained in culture. Under no conditions were the cells of the laminae able to grow out into protonema, the cultures being kept several months without any sign of growth. The costal portions after the usual length of time showed a growth of protonema in the ordinary way, only the number was greatly reduced. The cells of the lamellae are not able to grow out into protonema, neither when in connection with the leaf nor when separated. The power of regeneration is thus distinctly localized in the large cells of the costa lying at the base of the outer lamellae.

The whole plants which were kept under exactly the

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[1] Biolog. Centralblatt 13 : 647. 1893.

same conditions as the detached leaves gave rise to no protonema from the leaves. That the production of protonema was not called forth by cutting was shown by the experiments in which half of the leaf tip was cut away while the outer half was left in connection with the stem. Under these condition the portion of the leaf remaining in connection with the stem showed no growth. A complete separation of the leaf from the stem is then necessavy to call forth the power of the leaves to produce protonema and buds.

The defoliated stems of Atrichum when placed under conditions favorable to growth give rise to new shoots as axillary branches. This regeneration by means of new side branches occurred as readily in darkness as in light. In the dark the shoots grew more rapidly, producing more slender stems with reduced leaves. The tendency to apical production of shoots was not well marked, the shoots appearing at various points along the stem from base to apex. No new production of rhizoids or protonema were obtained from the stems under any conditions, although the cultures were kept for several months. Whole plants under exactly the same conditions as the defoliated stems produced new shoots as axillary branches but not in as great abundance as the defoliated stems, showing that the production is accelerated by defoliation.

POLYTRICHUM COMMUNE.

Cultures of leaves were made the same as for Atrichum, but it was not till the end of about six weeks that the growth of protonema was observed. The protonema were similar in nature to those already described for Atrichum, colorless walls and oblique cross-walls in both light and dark. A peculiar aggregation of branches occurred quite frequently an example of which is shown in Fig. 36, thus forming an assimilating organ, while the production of buds came later. In the protonema grown in the dark the cells were longer, without chlorophyll and the branching was more or less suppressed.

The protonema originated exclusively from the ventral surface of the leaf, that is the lamellae side, without reference to the position which the leaves occupied in culture. An examination of the leaves showed that the protonema came apparently from between the lamellae, but the exact origin could only be determined by means of cross sections. The sections showed that the protonema originated from the large cells lying just at the base of the lamellae. (Fig. 34) The protonema production did not seem to be confined to any particular portion of the leaf, but was quite generally distributed over the leaf cells occupying the position above named. The portion of the lamin a not covered by the lamellae is small, but the cells from that portion of the leaf as well as the cells of the lamellae were not able to grow out into protonema.

The first production of buds was noted at the end of about seven weeks, and the same as in Atrichum, in as great abundance in darkness as in light. The buds originated either as modifications of lateral protonema branches or in a few cases by the divisions of the end cell of a main protonema axis. The explaination for the production of buds in darkness, here as in Atrichum, is to be sought presumably in the nature of the leaf, the accessible supply of nutritive material being considerable.

The complete separation of the leaf from the stem was necessary to call forth the protonema formation. The experiments with the defoliated stems gave the same results as have already been dascribed for Atrichum. Mention may be made here of the cultures of Pogonatum nanum leaves. Cultures of these leaves were kept for two months without any appearance of protonema, although the leaves were apparently vigorous.

BRACHYTHECIUM RUTABULUM.

The leaves of Brachythecium did not produce protonema with very great readiness, only about one out of every eight giving rise to protonema. The first production of

protonema was noted at the end of nearly three weeks. As in the case of Bryum the first growth was neither distinctly protonema nor rhizoid. Even in the cultures in the light the main axis soon changed its cell-walls to a well-marked brown, while the side branches continued as distinctly protonema, with generally perpendicular cross-walls, and an abundant chlorophyll content. Buds were formed very soon and consequently the protonema did not attain any considerable size. In the cultures in the dark the filaments remained long and occasionally branched, with brown cell walls, oblique cross-walls and no chlorophyll. Buds were found after about three weeks, but these were confined to the cultures which had been exposed to the light. The buds originated in all cases as side branches of the main protonema axes.

The first experiments with the leaves showed that the protonema originated exclusively from the larger cells lying next the very base. None of the cells removed from the periphery gave rise to protonema, but only those one side of which was next the free lacerated base of the leaf. Leaves were cut transversely, and also longitudinally and all portions placed in conditions favorable for growth. The apical half of the leaf gave rise to no protonema whatever, while the basal half only produced protonema from the proximal end, with an origin the same as in the cases of the whole leaves. The portions of the leaves which had been split longitudinally also gave rise to protonema but only from the base and not from the cells lying along the cut margin. The power of regeneration is then located in the basal cells of the leaf the same as in Barbula muralis. The production of protonema is due to the separation of the leaves from the stem, since whole plants under exactly the same conditions as the detached leaves produced no protonema, and since when the y were cut and still allowed to remain in connection with the stem no growth was called forth.

Essentially the same results were obtained with leaves from a variety, except that the protonema originated exclu-

sively from the cells of the base which occupied the position next the costa.

The defoliated stems gave rise to new branches as axillary shoots, both from the main axes and from the side branches. These appeared without any apparent regularity with reference to base or apex of the stems, and also as well in the dark as in the light. In the dark the growth was more rapid, producing longer and slenderer shoots with very reduced leaves. Although the stems were cultivated for about two months no protonema production direct from the stems was observed. The production of rhizoids was not general only here and there a few being produced. The whole plants brought in the same conditions as the defoliated stems, produced new plants in apparently as great abundance. The rhizoid production was about the same, so it was impossible to say that either rhizoid or shoot production was accelerated by defoliation.

LEPTOBRYUM PYRIFORME.

The leaves of Leptobryum compare very favorably with B. rutabulum in the extent to which they produce protonema, perhaps not more than one in ten of the leaves used showing the formation of a new growth. The first appearance of protonema was noted after the leaves had been in culture for nearly three weeks. The same as in Bryum and Brachythecium, the first growth was semi-protonema in character. With continued exposure to light and increase in length, it assumed more and more the protonema character. Towards the distal end the cells were much shorter, abundantly filled with chlorophyll, and with perpendicular cross-walls. Even in the light the branching remained suppressed and only long, unbranched filaments of abont 1 cm length were produced. In the cultures in the dark the filaments remained distinctly rhizoid in nature and reached the length of about 1 cm after four weeks of growth. When leaves which had remained in the dark for about four weeks were

placed in the light, the continued growth of the rhizoids soon became of a more protonema character, so that a direct transformation of the main rhizoid axes to protonema was called forth. Even in this case no branching resulted and no buds were produced, neither in the light cultures nor in the dark.

The protonema originated from the basal portion of the leaf and generally from some of the cells a little removed from the end. There was no inherent tendency to the production of protonema from a particular side of the leaf. And, moreover, the growth occurred now from the contact side, now from the free side. In order to determine whether the cells removed from the base had the power of producing protonema, the leaves were cut transversely and kept in condition favorable for growth for about two months. No growth resulted from the apical portion, and from the basal portion the growth was the same as in the whole leaf. These experiments showed the power of growth to be localized in the cells of the basal half of the leaf. It was also shown that the production of protonema was only called forth by the complete separation of the leaf from the stem.

The defoliated stems when kept in culture a short time produced an abundance of rhizoids and protonema from the region of the leaf axils, the protonema generally being the more abundant. In the dark all of the growths from the stem retained the nature of rhizoids, but when exposed to light the further growth was of a decided protonema character. The protonema production was not local but occurred in as great abundance from one portion of the stem as another. The defoliated stems generally produced one or two new plants as lateral branches. The origin of these was not definite, since they appeared now at the base, now the apex and at intervening points. Whole plants placed under the same condition as the defoliated ones also produced au abundance of protonema direct from the leaf axil, the same as in the defoliated stems. The production of new shoots was also as abundant in the whole plants as in the case of the

defoliated stems. Hence, Leptobryum differs from the other species already described in that the protonema production is not called forth by defoliation.

The form of branching of the protonema of Leptobryum is worthy of note since it very frequently differs from the ordinary mode of protonema branching. In the normal branching of protonema each cell is able to form a branch just behind the cross septum. In this case however, two branches are formed opposite each other and immediately behind the septum (Fig. 48). The plants which I used for experiments were grown in the green-house, and an examination of the sterile plants showed that the production of protonema from the leaf axile was quite general. The side branches of these protonema often gave rise to strings of conidia-like cells, which broke away from the branch bearing them. The cells generally had assumed an oval form, were abundantly filled with chlorophyll bodies and quite often large oil drops and besides possessed slightly thicker walls (Fig. 43, 44). The striking similarity of this growth, to conidia formation in fungi will at once be noted from the diagram. Some of these conidia-like cells were placed in conditions favorable for growth and after a lapse of about eight days germination or growth had occurred as shown in Fig. 45. A great many of the leaf axils, instead of giving rise to protonema or rhizoids had produced dark brown, oval, multicellular brood-bodies borne upon a stalk several cells long (Fig. 46, 47). The rhizoids also gave rise to similar brood-bodies. The conditions for this conidia production can not be stated. In the artificial cultures when kept moist, this manner of breaking up of the protonema branches into single cells did not occur. The plants in the green-house were not however especially dry.

PHASCUM CUSPIDATUM.

The leaves of Phascum produced protonema with great readiness and in less time than any other species investigated. Cultures were made for both light and dark, part of the

leaves in each case being dorsal side and part being ventral side up. At the and of five days both protonema and buds had been produced in the light, and a careful examination showed that the majority of the growths originated from the ventral side of the leaf in the region of the costa, without reference to the position which the leaves had occupied in the culture. Occasionally a protonema originated from the leaf cells removed from the region of the costa, and now from the contact side and now from the free side of the leaf. Occasionally some of the cells from the region of the base gave rise to distinct rhizoids which showed no tendency to produce protonema branches, but remained distinctly rhizoid in nature even in the light. The branching of the protonema in the light was not very profuse, while in those which remained in the dark all the time, the branching was suppressed to a considerable extent, and the walls very soon turned brown. No buds were formed in the dark cultures, while the cultures in the light had plenty of buds at the end of five days.

When the lateral halves of the leaves were separated from the costa, they also gave rise to numerous protonema showing that in the whole leaf the ability of regeneration was present, but that the supply of food material was contributed to the cells of the costal region, which produced protonema with greater ease. It has abready been stated that the majority of protonema production in the whole leaves was from the ventral leaf surface in the region of the costa. Cross sections of the leaves showed that the cells occupying the costal region on the ventral side were thinner walled than the remainder of the leaf cells, hence they produced protonema more readily. The dorsad side of the costa was made up entirely of thick walled cells, hence no protonema production from from the dorsal side occurred. (Fig. 51.)

Whole plants and plants with the tips of the leaves cut away produced no protonema from the leaves, nothing but a complete separation of the leaves from the stems being

sufficient to call forth the formation of protonema. The protonema production from the costal region occurred throughout the entire extent of the leaf, but the number from the apical portion was greater than that from the basal portion.

The defoliated stems produced protonema direct, throughout their entire length, and they were not confined entirely to the leaf axil, any of the surface cells being capable of growth. The defoliated stem generally produced at least one new shoot, and sometimes this originated from the very base of the stem, sometimes from nearer the apex. The whole plants placed in exactly the same conditions produced protonema direct from the stem, and also new shoots.

CERATODON PURPUREUS.

All attempts to obtain protonema from the leaves of Ceratodon were without effect. The leaves were kept for several months in apparently vigorous condition without any sign of protonema formation. The defoliated stems are however able to give rise to an abundant protonema growth, which originates direct from the region of the leaf axils. Rhizoids are also produced but they very soon became protonema in nature in the cultures which are exposed to light. In the dark the growths were more of a rhizoid nature and generally remained almost devoid of branches. The protonema production was not local but was general throughout the entire length of the stem. The defoliated stems also produced new shoots as lateral branches. The point of origin was not definite, since they might come at any point between the base and apex of the stem. The production of new shoots occurred as well in darkness as in light. Whole plants placed in the same conditions as the defoliated stems, produced new lateral shoots and quite a number of protonema, hence the protonema and shoot production was not called forth by defoliation.

FISSIDENS BRYOIDES.

The same as in Ceratodon, all attempts to grow protonema from the leaves of Fissidens were ineffectual. The leaves were kept for three months and at the end of that time, although in apparent vigor, no protonema had been produced. The stems, when stripped of leaves, produced rhizoids direct from the region of the leaf axils in both light and darkness. In the light howerer the rhizoids soon grew to possess a distinct protonema character, but no protonema originated direct from the stem. The stems grown in the dark produced long, sparcely branched rhizoids, which attained a length of about 1 cm after a month of growth. When first examined they possessed only oblique cross-walls, but at this time nearly all showed alternately oblique and perpendicular walls. The oblique walls were the ones first formed and the perpendicular walls were produced later by intercalary division. The great regularity of the alternately oblique and perpendicular cross-walls was due to the fact that each cell had become divided by a perpendicular wall. This fact is mentioned since intercalary division is an exception to the usual mode of protonema and rhizoid growth, and since it affords another example of perpendicular cross walls being produced in darkness.

No buds were produced from the protonema grown from the stem, but the stem gave rise to buds, and that in a peculiar way. After one month of culture the stems grown in the light were found to have produced buds direct from the region of the leaf axils, without the intervention of any protonema. A bud grown in this way is shown in Fig. 53. A surface cell from the region of the leaf axil produces a protuberance, which instead of growing out into a rhizoid or protonema divides directly to form a bud. This manner of bud formation was observed only in light cultures. Plants with the leaves still in tact, also produced buds in the same way although not in as great abundance as in the defoliated stems. The buds were in the course of time detached from

the stem. This manner of bud formation is of interest as affording another example of the production of buds without the intervention of a protonema. It is very probable that buds are produced this way in nature, and the presence of young plants coming from the region of the leaf axil conforms the supposition. The direct growth in nature was not followed however. Mention may be made here of the attempts to obtain protonema from Fontinalis antipyretica. The leaves and stems were cultivated in a variety of ways: in water, on earth, and with varying amounts of moisture, but no protonema were obtained from either leaves or stem.

As shown by the foregoing experiments, the production of buds with reference to light and darkness seems to have been in a great measure dependent upon the supply of food material which the leaf could afford. The question which naturally suggests itself at this point is: Can bud production be called forth in the dark by the use of some such carbohydrate food as grape-sugar, in the case of leaves which in themselves are unable to produce buds with the absence of illumination? This is a question difficult to solve, because the majority of leaves require a considerable length of time for bud production, and because it is impossible to make perfectly sterile cultures. Repeated attempts were made with various leaves, with every care possible to keep the cultures sterile, but the inroads of bacteria and moulds destroyed the experiments and thus shut out all chance of success.

In one instance however my efforts were suocessful and that in the case of Phascum cuspidatum. The leaves of this moss under ordinary conditions produced protonema and buds after five days. The rapidity of growth made it favorable for experimentation and the cultures to which grape sugar was supplied, formed buds after two days, both in light and darkness, but in greater abundance in the light cultures. With further growth in the dark, the buds grew

to produce shoots, two, three, and in one case five millimeters in length.

Experiments were performed with several of the species which produced protonema the most readily to see what effect KNO_3 would have upon the regeneration and manner of growth. Barbula muralis and Phascum cuspidatum leaves were grown in 1 % KNO_3 without any apparent retardation or change in the manner of growth. Bryum capillare leaves produced protonema, but there was a marked retardation of growth and the filaments did not reach any considerable size. In 2 % KNO_3, Barbula muralis still produced a vigorous growth without any marked retardation. The cells were however generally shorter, and the branching more aggregated. Bryum capillare and Phascum cuspidatum produced no growth whatever. In 3 % KNO_3, Barbula muralis produced a slight growth but the filaments did not reach any considerable length.

A series of experiments was also carried out in order to determine the temperature at wich protonema formation would occur. For these experiments Barbula muralis, Bryum capillare, and Phascum cuspidatum were used, with the results given in the following table.

	19—21°	24°	27°	29,5°	32°	36°
Barbula muralis . . .	×	×	×	×	×	
Phascum cuspidatum .	×	×	×	×	×	
Bryum capillare . . .	×	×	×			

Barbula and Phascum produced protonema with as great vigor as 32° at at 19—21° C, the temperature of the ordinary experiments; but at 36° C no growth resulted. At 29,5° C the Bryum leaves produced no growth but were not killed since when exposed to the ordinary temperature, protonema were produced. At 32° C however, the leaves were killed. At 27° C a slight growth resulted but with a very marked retardation. At 24° the growth was to all appearances quite normal.

That moss plants are able to be dried completely for some length of time and still retain their power of regeneration has been demonstrated by Schröder.[1] By way of confirmation, Bryum capillare was dried thoroughly for three weeks, then moistened and the leaves stripped from the stems and placed in conditions favorable for development. In the same time as usual protonema made their appearance. Barbula muralis was dried for two weeks without the loss of protonema production.

The foregoing experiments have shown that in nearly all conditions, the only requisite for the development of protonema from rhizoids has been the exposure to light. Either the main rhizoid axis has given rise to side branches which were distinctly protonema in nature, or the continuation of the main axis has become decidedly protonema like. There may, however, be conditions in which the rhizoids even though exposed to light do not produce protonema branches. The rhizoids from Mnium leaves in case the normal development of buds is allowed to be carried out produce no protonema branches. In the same way the rhizoids from the stem did not give rise to protonema branches, but if the growth of the stem is interrupted the rhizoids undertake the regeneration of the plant and produce new leafy shoots, and protonema branches. This manner of growth is quite common when tufts of various plants are inverted so that the rhizoids are exposed to the light and the shoots killed by being covered with soil.

The experiments which I have carried out show that the protonema do not produce rhizoids with as great readiness as the rhizoids do protonema. This is in opposition to the view expressed by Frank,[2] since he says in regard to the protonema: "Eben so leicht kann der Faden wieder in ein Rhizoid sich umwandeln." A protonema of Bryum capillare was grown on a piece of flower pot until a considerable size

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[1] Untersuch. aus d. Bot. Inst. zu Tübingen 2 : 15—21. 1886.

[2] Lehrbuch der Botanik II : 9. 1893.

and vigor was attained, and then placed in the dark. At the end of two weeks no sign of rhizoids was detected. The growth had however been considerable from the supply of food material which had been produced in the light.

In another case a luxuriantly growing protonema of the same species as above mentioned was placed upon a piece of flower pot and one half covered with earth, the other allowed to remain free. Only in one or two cases was a growth of rhizoids noted from the part covered with earth. The same result was obtained with protonema of Bryum capillare and Barbula muralis in which one half was covered with a screen of black paper. The protonema lost their chlorophyll content, bud did not develop any distinct rhizoids. From these results it is seen that although exposed to darkness and also grown on earth, a rhizoid production only rarely occurred. A culture of protonema of Barbula muralis which was grown in the light produced distinct rhizoids after about eleven weeks of growth. Here then is a case of the production of rhizoids in direct illumination. Bryum capillare and Barbula muralis leaves were grown under water and a luxuriant protonema growth obtained. It might be thought that growing under these conditions, the protonema would retain their more algal nature, and not produce new leafy shoots; but, in the case of Bryum buds made their appearance after the usual length of culture. There was however a difference in the form of growth. In Bryum and Barbula the lateral branches grew quite slender and tapering, while in the cultures on flower pots they were more robust and of equal diameter throughout. In Barbula these side branches frequently possessed oblique cross-walls, while Bryum generally had perpendicular cross-walls. This manner of growth has been mentioned by Goebel[1] for a protonema of Physcomitrium pyriforme when grown in water. He compares these side branches to rhizoids and makes the statement that they evidently correspond to rhizoids. It might be

[1] Flora 72:8. 1889.

infered that the lack of rhizoid production in these cultures was due to the medium of growth, either upon flower pot pieces or in water. Cultures which from the beginning were made upon earth showed essentially the same manner of growth, except that the side branches were robust instead of slender, of equal diameter instad of tapering and were distinctly positive heliotropic. It was only very rarely that a protonema branch was found penetrating the soil and becoming of a rhizoid nature. The same result was obtained with protonema which were grown either in water or upon flower pot pieces and then placed upon the soil, the further growth still being without rhizoid development. Luxuriantly growing protonema from the stem of Funaria were half covered with earth without any appearance of rhizoids. Schimper[1] grew Funaria protonema from the spores which did not show any rhizoid production.

SUMMARY.

Considering the various species of moss plants used in the forgoeing experiments, there are, notwithstanding the variety of results, many striking similarities in the manner of regeneration, a brief summary of which will be brought together in the following conclusions:

1. The majority of moss leaves used showed a remarkable power of regeneration, producing either rhizoids or protonema, with the later appearance of new leafy shoots. The rhizoid or protonema production was carried out in both light and darkness.

2. The point of origin of the new growth from the leaf in some cases depended upon contact and illumination and was independent of gravity (Mnium). In other cases the protonema had a definite origin which was independent of external factors, and depended solely on the leaf structure: from the ventral side of the leaf as in Atrichum, Poly-

[1] Rech. anat. et morph. sur les mousses, Plate I. 1848.

trichum and Phascum; from marginal cells and thus independent of contact, gravity, illumination, or position of the leaf.

3. The power of regeneration may be distintcly localized: *a.* In special cells of the leaf base as in Barbula, Brachythecium, and Funaria. *b.* In special cells of the ventral leaf surface as in Atrichum and Polytrichum. In other cases the power of regeneration was quite generally shared by all the leaf cells as in Mnium, Bryum, and Phascum.

4. The structures produced might be entirely rhizoid in both light and dark as in Mnium, and occasionally so in Phascum. They were protonema in light and rhizoids in the dark as in Bryum, Barbula, Brachythecium and Phascum, or they were entirely protonema in both light and dark as in Atrichum and Polytrichum.

5. Buds were produced under ordinary conditions of cultivation only in the light in the following: Mnium, Funaria, Bryum, Barbula and Brachythecium. In both light und dark by Atrichum and Polytrichum under ordinary conditions and by Phascum when supplied with grape sugar. The production of buds seemed to be in a measure dependent upon the food supply.

6. The regeneration was called forth in all cases by the separation of the leaf from the stem. The mere cutting of the leaves while in connection with the stem did not call forth the production of protonema or rhizoids.

7. The majority of moss stems as well as the leaves showed regeneration and that in two ways: *a.* By axillary shoots. *b.* By protonema direct or by rhizoids which in the light very soon gave rise to protonema branches. The stems in two cases had the power of regeneration while this power was not shared by the leaves (Fissidens and Ceratodon).

8. The production of axillary shoots was not called forth by defoliation of the stem, but was generally accelerated thereby. In some cases the protonema production was called forth by defoliation, in other cases only accelerated.

9. The protonema production was quite general throughout the entire extent of the stem. In some cases the pro-

tonema originated only from the axillary cells, in other cases from the various surface cells of the internode. The axillary shoots in one case showed a tendeney to marked apical origin (Mnium). In the other cases the distribution was quite general.

10. The buds originated from Mnium leaves and Fissidens stems without the intervention of a protonema. When of protonema origin, they were either modifications of lateral protonema or rhizoid branches, or direct modifications of the main axes. The tendency of protonema to produce rhizoids was not as great as the tendency of rhizoids to produce protonema.

11. The upper temperature limit for regeneration from the laves investigated varied from 24 to 32° C. Protonema were grown in 1 and 2% solutions of KNO_3. Drying for a considerable length of time did not alter the power of the leaf to produce protonema.

THE EFFECT OF LIGHT UPON THE GERMINATION OF CRYPTOGAM SPORES.

1. INTRODUCTION.

The investigations upon the effect of light on the germination of fern and moss spores have led to opposite and contradicting results. According to Borodin, Schmidt and others, the failure of fern spores to germinate in the dark is experimentally demonstrated, while Göppert and Schelting arrived at exactly opposite conclusions. Leitgeb has shown the necessity of light for the germination of liverwort spores, and Milde succeeded in germinating Equisetum spores in the dark. Up to this time no systematic work on the germination of moss spores in light and darkness has been carried out. In order to clear up this existing confusion and extend our knowledge in regard to the conditions for the germination of moss spores, the present investigation has been carried out.

Before proceeding with the results of my own experiments however, I will treat a little more in detail the investigations bearing upon this subject, which have been hitherto published.

2. HISTORICAL.

The early botanists were in no sense of the word physiologists, and so from the time when the spores of mosses were first observed and compared to the seeds of flowering plants down to almost the present time, their germination has been treated almost exclusively from the the morphological point of view. A historical summary of the works on

the germination of the spores of Musci and Hepaticae up to 1884, is brought together by Lindberg.[1] The summary is not quite complete, as no mention is made of the result which Borodin[2] obtained with spores of Polytrichum commune. He found that they were unable to germinate in darkness. The work of Müller-Turgau[3] on the germination of spores and the production of secondary protonenma is also omitted.

As regards the fern spores, the earlier investigators made the assertion that light prevents their germination, as is to be noted in the works of Senebier, Humboldt,[4] Ingenhauss,[5] and Treviranus.[6] More recent investigators as Kaulfuss,[7] Leszczye-Suminski,[8] Merklin,[9] Wiegand[10] and Hofmeister[11] intimate that light is one of the necessary conditions for germination, although no definite investigations in in that line are mentioned.

The first investigations of importance from the physiological point of view are those of Borodin.[12] He experimented with eight different species and found that in all cases light was necessary for germination, and that in the dark, no bursting of the extine occurred. His experiments are lacking in one thing, since he does not state at what temperature the cultures were kept. As shown by my own investigations this is one of the most important points. Two years later Göppert[13] succeeded in bringing the spores of Os-

[1] Historiska data Rörande var Kännedom om Moss-sporens groning. Helsingfors, 1884. Rectorprogram.

[2] Bull. de l'acad. imp. de S. Petersbourg, 12:433—440. 1867.

[3] Arb. d. Bot. Inst. zu Würzburg, 1:475—499.

[4] Aphorismen, p. 90.

[5] Versuche mit Pflanzen II. 5. Abschnitt.

[6] Physiologie der Gewächse. 2. II. Abth. 584. 1838.

[7] Das Wesen der Farnkräuter pg. 59. 1827.

[8] Zur Entwickelungsgeschichte der Farnkräuter p. 8. 1849. Berlin.

[9] Beobachtungen am Prothallium der Farnkrauter p. 5. 1850.

[10] Entwickelungsgeschichte der Farnkräuter. Bot. Zeitung. 7:17. 1849.

[11] Vergleichende Untersuchungen p. 78. 1851.

[12] l. c. p. 529—541.

[13] Schmidt, Über einige Wirkungen des Lichtes auf Pflanzen. p. 21. 1870, Breslau.

munda to germinate in the dark, but the temperature at which the cultures were grown is unknown to me. A year later, Schmidt,[1] with cultures of the spores of Aspidium violacens and felix-mas, confirmed the results previously obtained by Borodin. In 1872 Kny[2] obtained results which contradicted those of Göppert for Osmunda spores. The next work of importance was that of Schelting[3] in 1875. He worked with the spores of four different species and found that in all cases germination occurred in the dark. One of the species which he used, Aneimia Phyllitides, was also used by Borodin in the invettigations above cited. I have not had access to the original paper, but the probability is from the review, that the cultures were kept at a temperature higher than the normal room-temperature.

Again later, G. Beck[4] has shown that the spores of Scolopendrium vulgare germinate only when exposed to light.

Milde[5] and Sadebeck[6] have shown that the spores of Equisetum germinate in the dark as well as in the light, while Leitgeb[7] in his excellent work on the liverworts has shown that darkness prevents the germination of the spores; also that faint illumination causes the development of protonema which differ markedly in form from those grown under normal illumination. With this short historical summary as an introduction I will proceed to the results of my own investigations.

3. EXPERIMENTAL.

The same method was used as has been previously described for the experiments with moss leaves: either filter paper in Petri-dishes, or pieces of flowerpots, or sterilized earth. Any

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[1] l. c. p. 20.
[2] Jahrb. f. wiss. Bot. 8:4. 1872.
[3] Bot. Jahresber. 1875, 328.
[4] Bot. Zeitung, 36: 780. 1878.
[5] Nova Acta Acad. L. C. F. 23:2.
[6] Bot. Zeitung, 35:44 and 45. 1877.
[7] Sitzungsber. d. Akad. d. Wiss. Wien. 74:1. 1876.

special methods will be described in connection with the experiments themselves.

a. MOSS SPORES.

First as to the experiments with moss spores. Cultures of Funaria hygrometrica spores were made, and in one case exposed to normal illumination, and in another placed in the dark chamber; both being kept at a temperature varying from 19—21° C. At the end of three days the spores exposed to light had germinated abundantly, while those in the dark chamber showed no signs of germination, not even a bursting of the exospore. The dark culture was kept for a month and at the end of that time there was no indication of germination. That the spores had remained normal and had not lost their power of growth was shown by their speedy germination when exposed to ordinary illumination. This experiment was repeated several times with the same result. Similar experiments were carried out with spores of Brachythecium rutabulum, Bryum pendulum, and Mnium cuspidatum and all revealed the same dependence of germination upon illumination.

In order to determine which part of the spectrum was effective in producing germination, cultures of spores were placed under double-walled bell-glasses filled respectively with potassium bichromate and ammoniated copper oxide. The cultures included Funaria hygrometrica, Bryum pendulum and Brachythecium rutabulum. At the end of three days the spores exposed to the less refrangible rays of the spectrum had germinated with as great readiness as under normal illumination, while the cultures in the blue light showed no signs of germinating, thus acting the same as in darkness. The spores although retained in the blue light for over a month showed no germination whatever. The failure of the spores to germinate in the strongly refrangible rays would seem to throw some light upon the processes which occur in germination. Although the spores form some chlorophyll in the blue light, the photosyntactic processes are not active,

and hence it might be thought that germination depended upon the elaboration of new material which can only occur to any extent in the less refrangible rays. That this view is highly improbable is shown by the experiments which follow.

Cultures of spores were made of the three species above mentioned and placed in CO_2 free air in the light, the apparatus being the same as that already used for the moss leaves. The first series of experiments in bright light showed that photosyntax was not necessary for germination, since the spores had germinated as readily in the CO_2 free air as under ordinary conditions. The same result was obtained when the apparatus was exposed to less intense illumination by the interposition of an opaque screen. Under these these conditions the photosyntax would be insignificant and hence it appears evident that germination is independent of that process. The influence of light in germination must be sought then, presumably in a transformation of food products already present in the spore; these chemical changes being initiated by light and only by the less refrangible rays. More in regard to the nature of this transformation will be brought forward when later experiments are described.

The question which now presented itself was: Is continued exposure to light necessary for germination, in other words, in there a light induction? In order to determine this point cultures of spores which had been in the dark for twelve hours, were placed in the light and carefully watched for the first signs of germination. After about fourteen hours of illumination the spores showed the first signs of germination, in some cases the extine being burst; a slight protuberance the beginning of the protonema also being evident. Half of the cultures were allowed to remain in the light as control experiments, while the other half were removed to the dark chamber to undergo further development. Those spores which showed no beginning of germination before removed to the dark, did not germinate in the dark although they had formed an abundant chlorophyll

content. Those spores which had begun to put out a protonema filament, continued their growth somewhat, but the filament was long and slender and did not attain any considerable size on account of lack of plastic material. These experiments were carried out with spores of Funaria hygrometrica, Bryum pendulum, and Brachythecium rutabulum, all with the same result. Essentially the same fact has been shown by Borodin[1] for fern spores.

Leitgeb[2] has shown that for the germination of liverwort spores, a certain intensity of light is necessary, and my experiments with moss spores show that the same thing is true only to a less marked extent. In experiments which I conducted with Marchantia polymorpha spores, parallel with the cultures of moss spores, where all were exposed to the light in the middle of the laboratory, the different behavior was very marked. The moss spores germinated in the usual length of time and without any apparent modification due to the weakness of the light. The Marchantia spores on the other hand showed a very remarkable retardation in germination, and when germination did occur, only a long narrow filament was produced which gave no indication of the formation of the thallus according to the ordinary method of growth in sufficiently intense illumination. Other cultures were made and exposed to a much weaker light, a room in the Institute basement with only one window being used. First, cultures were placed on a shelf at a distance of about three meters from the window, then at two meters, and then in the window itself. The cultures at a distance of three and two meters from the window, showed a complete failure of the spores to germinate, although they produced chlorophyll to some extent. Those which were grown in the window, germinated after the ordinary length of time. That the spores remained capable of germination was shown by the fact that they began growth as soon as they were exposed to normal illumination. These experiments then

[1] l. c. p. 539.

[2] l. c.

show that under ordinary conditions of temperature and food supply, the moss spores require a certain intensity of light for germination, but that the required intensity is not as great as in the case of the liverwort spores. These facts were demonstrated for the three species of mosses mentioned above.

Reasoning from the results which I had already obtained with fern spores, a series of experiments was carried out in which cultures of spores were exposed to different temperatures. The failure of the spores to germinate in the dark is due as has already been stated to the fact that the conditions of temperature, light, etc. were such that certain chemical processes necessary for germination could not be active. The results with fern spores show that heat is able to effect this change as well as light, so that germination may be called forth in complete darkness by subjecting the spores to a higher degree of temperature than the normal room temperature. That moss spores would be affected in the same way as fern spores would seem quite probable, but nevertheless my experiments in this direction have failed to find any temperature at which moss spores will germinate in complete darkness, when supplied with only inorganic material. The temperature to which the general cultures was exposed ranged from 19—21° C. Cultures were made for the following degrees of temperature: 23°, 24°, 27°, 29°, 32° and 35° C and in each case in complete darkness. The cultures at 35° C were kept for four days. At the end of that time no signs of germination were visible, hence they were removed to the light and at the ordinary temperature. The failure of the spores to germinate under these conditions, showed that they had been killed by the high temperature. The other cultures were allowed to remain in dark for six days and then removed to the light. The spores subjected to 32° were not killed, but a very marked retardation of germination occurred, since the period required for germination was extended from three days to ten. The cultures that had been kept at 29° also showed a retardation of germination,

five days being required after the exposure to light. In the other three series of cultures at 27°, 24° and 23°, no apparent retardation of germination was noticed when the spores were exposed to light.

The above experiments have shown clearly that a continuous exposure to high temperatures is not sufficient to produce germination of the moss spores in the dark. As suggested by the results obtained by Liebenberg [1] for seeds of Poa, it was thought that perhaps a *change* of temperature might be effective in producing germination. To this end the following experiments were carried out. Two cultures of Funaria hygrometrica spores were placed in the dark for twelve hours, then in the thermostat at 41° C, for four hours. The cultures were then removed and one placed in light, the other in the dark, both at a temperature of 19—21° C. After three days the spores in the light had germinated abundantly, but those in the dark showed no signs of growth, although they were kept for two weeks. Similar cultures were exposed to a temperature of 41° C for three hours with the same result.

Cultures similar to the above were made for Funaria hygrometrica, Bryum pendulum, and Brachythecium rutabulum and exposed to a temperature of 32° C for twenty four hours. At the end of this time they were placed at the ordinary temperature, the control experiments in the light, the others in the dark. Those in the light germinated after the usual length of time, but in the dark no signs whatever of germination were noted. Thus, change of temperature is also shown to be insufficient in producing germination in complete darkness.

It is known that ether has a stimulating influence on the production of shoots from certain phanerogams, when under normal conditions none are produced. It might also be supposed that it would act as a stimulant to call forth the germination of spores in the dark. In order to determine this point, a series of cultures was made in which the spores

[1] Bot. Centralblatt **14**: 21—26. 1884.

were subjected for different lengths of time to a saturated or partially saturated atmosphere of ether. Cultures of Funaria spores were allowed to remain in the dark for twenty-four hours, in order that they might be in a moist condition, and then placed in an ether atmosphere. In the first case they were exposed to the ether atmosphere for one hour, in the next two hours, and in the next three hours. Two cultures were used in each case and as soon as they were removed from the ether atmosphere, one was placed in the light and the other in the dark chamber. The control experiments in the light showed that in the experiments to which a two and three hours exposure to ether was given, that germination did not take place, and hence that the spores had been killed by the strong ether atmosphere. In case of the spores which had been in the ether for one hour, germination occurred in the light, but it was considerably retarded. In the dark no sign of germination was noted.

From the above experiments it was quite evident that too strong a dose of anaesthetic was administered, so another series of experiments was conducted in which the spores were subjected to an atmosphere containing less ether. In order to supply the ether atmosphere, 1 part of ether was mixed with 20 of water. The cultures were then exposed to this atmosphere for one and three hours respectively. Those spores which had been in the ether atmosphere for one hour, showed a very slight retardation of germination even in the light, but in the corresponding dark culture, no germination whatever was noted. Those spores which had been in the ether atmosphere for three hours, showed a very marked retardation, the period required for germination being extended from three to ten days. Those in the dark showed no germination. Another culture was treated in a slightly different way; it was placed in the ether atmosphere for one hour, then in the dark for twenty-four hours, then in the ether atmosphere again for one hour, and from that time on, in the dark under ordinary conditions. These cultures were kept in the dark for three weeks with the complete failure of the spores to

germinate. In so far as the above experiments are concerned, ether retarded the germination of the spores even in the light, and had no affect upon their germination in the dark.

The non-nitrogenous food supply of spores is in the form of oils or fat. The first change of the fats in germination is apparently a decomposition into glycerine and fatty acid.[1] That the ultimate product from this food supply which is used in the first is a carbohydrate in the form of sugar may be surmised. At any rate the failure of the spores to germinate in the dark is due presumably to the fact that conditions are not afforded for the chemical changes which the reserve material must undergo before it can be used as plastic material for the growth of the cell. There is a possibility that the failure to germinate may be due to the proteid reserve material remaining in a form which can not be used. This is howerer not as probable as the view just advanced for the non-nitrogenous food reserve. If the supposition is correct, spores when supplied with organic material in the form which the reserve assumes ultimately in germination, might be expected to germinate in complete darkness.

As a nutritive solution the following preparation was made: to 100 cc of $^1/_4$ pro mille normal, inorganic nutritive solution, 2% of grape sugar and one percent of peptone was added, and the whole sterilized on the water bath for one hour. Cultures of Funaria spores were made for both light and dark and supplied with this nutritive solution, as great precautions as possible being taken to keep the cultures sterile. An examination of the cultures at the end of three days, showed that the spores had germinated as well in the dark as in the light. The very noticable feature of this experiment was that under these conditions the protonema were four or five times as large as when supplied with only inorganic nourishment and grown in the light; also that the cells were crowded with large, irregular starch masses, as shown by the iodine test. The question now was: Is this

[1] Vines, Physiology of plants p. 173. 1886.

germination in the dark due to the sugar or the peptone or both? In order to determine this point, the following experiments were carried out.

A $2^0/_0$ grape sugar solution was made from the $^1/_4$ pro mille normal nutritive solution, and cultures of the Funaria spores made for both light and dark. After three days an examination of the cultures showed that germination had occurred as well in the dark as in the light, thus demonstrating the power of grape sugar alone to call forth germination in the dark. A one percent peptone solution was then prepaired in the $^1/_4$ pro mille normal nutritive solution, and cultures of Funaria spores made for both light and dark. After three days, these cultures also showed germination of the spores in both light and darkness, with the same increase in size of the protonema as in the case where sugar alone was used. In the cultures with sugar nearly every spore germinated, both in light and in darkness, in the peptone culture in the light also the same, but in the dark the number of spores which germinated was relatively small. The above results had already been obtained when Goebel's preliminary note[1] concerning the same phenomena appeared.

Similar experiments to the above were carried out for Bryum pendulum, Brachythecium rutabulum, and Mnium cuspidatum. With these species essentially the same results were obtained as regards the germination in light and darkness, but the protonema showed no increase in size, which was such a noticable feature in the case of the Funaria spores. In the peptone cultures the number of spores germinating in the dark was rather smaller than for Funaria.

That the germination in the dark is due to the nutritive value of the sugar and peptone is highly probable, but still it might be claimed that osmotic pressure was the active agent. In order to throw some light upon this point, the following experiments were carried out. Spores of Funaria hygrometrica, Bryum pendulum, and Brachythecium rutabulum

[1] Flora 82: 75. 1896.

were placed in culture and supplied with a 0.5% solution of KNO_3. The cultures in the light showed germination after the usual length of time, but no sign of germination was observed in those which were deprived of light. Experiments with the same results were also carried out for the same species in a 1% solution of KNO_3. De Vries[1] has shown that the osmotic value of KNO_3 is about double that of grape sugar with equal parts of the gram molecule, or more exactly, the isotonic coefficient of grape sugar is 1.88. The osmotic value of a 0.5% solution of KNO_3, or approximately a $^1/_5$ gram molecule solution, would be about the same as that of a 2% grape sugar solution, or a $^1/_9$ gram molecule. The failure then of the spores to germinate under the above conditions would tend to show that the osmotic pressure within the spore could not have been the operative force in bringing about germination.

The absence of any effect from osmotic pressure was also rendered probable from the experiments in which the spores were supplied with either glycerine or potassium tartrate. Spores of the three species above mentioned germinated readily in one and two percent solutions of glycerine in the light, but in the dark they remained unchanged. Glycerine is a non-nutritive substance for the moss-spores and at the strength used would be about osmotically equivalent to the sugar. In the 1% solution of potassium tartrate, the spores germinated neither in the light nor dark, but in the 0.5% solution the growth was the same in the light as in the control experiment. In the dark cultures supplied with 0.5% potassium tartrate there was a complete failure to germinate. The isotonic coefficient of potassium tartrate is 3.99[2] and consequently the osmotic value of the last solution would not be far from that of 2% grape sugar. Cultures of spores which were supplied with a 2% solution of lactose, also non-nutritive for the moss spores, showed the

[1] Jahrbücher für wissenschaftl. Bot. 14: 454. 1884.

[2] De Vries l. c. p. 506.

same failure of germination in complete darkness. The spores which were exposed to light germinated however with as great readiness as in the control experiment, where they were exposed to ordinary conditions.

It is known that certain substances like iron chloride and cobalt salts, when used in a solution which is too dilute to be poisonous, exercise an accelerating influence upon the growth of fungi. These substances are non-nutritive and the acceleration of growth is presumably due to a so-called catalytic action. This fact suggested the possibility of calling forth germination in the dark by means of such substances, and to this end the following experiments were performed. Spores of the species generally used were grown in different strengths of iron chloride: 0.25%, 0.125%. In no case was germination called forth in the dark. In the 0.25% solution the Bryum and Brachythecium spores germinated neither in light nor darkness. In the 0.125% solution however, the spores germinated abundantly in the light. A series of experiments was also carried out in which spores of Funaria hygrometrica were supplied with a dilute solution of cobalt sulfate, C_0SO_4. I have shown in my investigations with seedlings[1] that cobalt solutions are extremely poisonous; hence in order to obtain solutions which would not have a toxic action, a very great dilution of the stock solution was required. Sowings of the spores were made for both light and darkness and supplied with $\frac{1}{10000}$, $\frac{1}{20000}$, $\frac{1}{40000}$, and $\frac{1}{80000}$ gram-molecule solutions. In all of the cultures the spores germinated in the light without any marked retardation, but in the dark, the same as in previous experiments, no germination occurred. Thus all of the previous experiments point to the fact that germination in the dark was due to the nutritive value of the sugar and peptone and not to any stimulating or catalytic action.

It is also interesting to know the minimum amount of sugar which will suffice to call forth germination in the dark.

[1] Bot. Gaz. 22 : 143. 1896.

First, cultures of Funaria spores were supplied with $\frac{1}{900}$, $\frac{1}{225}$ and $\frac{1}{135}$ gram molecule solutions of grape sugar and placed in darkness. An examination after three days showed that in the first two dilutions, none of the spores had germinated, while in the $\frac{1}{135}$ gram molecule solution they had germinated the same as in light under ordinary conditions; and also with the usual increase in size, and with the accumulation of starch. The spores of Bryum pendulum also germinated in a solution of the same dilution, but those of Brachythecium required a still stronger solution, only germinating in the dark when they were supplied with $\frac{1}{90}$ gram molecule. The maximum concentration at which germination can occur is not so important but results were obtained in this line for a single species. Cultures of Funaria spores were supplied with 5, 10 and 20% solutions of grape sugar. The first two concentrations allowed germination in both darkness and light, but in the 20% solution the spores germinated neither in light nor darkness. In the 5 and 10% solutions, the protonema which were formed in the light were perfectly colorless and without chlorophyll.

The great difficulty of obtaining perfectly sterile cultures of moss protonema upon an organic substratum will at once be evident to all who have ever worked in this line. Goebel[1] was unable to obtain perfectly sterile cultures in his investigations upon Funaria hygrometrica. If perfectly sterile cultures could be obtained, it would be possible then to determine whether moss protonema are able to thrive in the dark, when supplied with organic material as sugar and peptone. This was the problem which now presented itself for solution and to which my attention was next directed. A considerable number of attempts were made and at last my efforts met with success. The details of the experiments I will describe in the order in which they were carried out.

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[1] Goebel, Sitzber. d. mat.-phys. Classe d. k. Bayer. Akad. d. Wiss. 26 : 462. 1897.

The medium for the growth of the spores was made as follows: —200 cc of $^1/_4$ pro mille normal nutritive solution.

2 gr.—grape sugar,
1 gr.—peptone,
1 gr.—agar-agar.

The mixture was boiled on a water bath for three hours and then filtered, and preparations made in small Petri-dishes.

Capsules of Funaria were selected which had the operculum still in tact and attempts were made to sterilize them. They were first soaked in water until the water had penetrated them thoroughly and then placed in $1^0/_0$ formol for different lengths of time. The preparation of the cultures was carried out under all possible precautions against infection, in a chamber which had been saturated with steam. As far as these experiments were concerned it was shown that an immersion in the $1^0/_0$ formol for a length of time sufficient to kill the adhering germs, also proved fatal to the spores.

In order to preserve the spores and at the same time have the capsules in a sterile condition, I was obliged to resort to another method. The capsules were first dipped in melted parafin and perfectly coated over, and then placed in the formol. The coating of parafin thus prevented all penetration of the formol into the interior of the capsules. Then, by operating in the chamber which had been saturated with steam, perfectly sterile cultures were prepaired.

The Petri-dish cultures offered subsequent opportunties for the penetration of moulds, even when extreme care was taken, so that cultures which had been kept sterile for several weeks would often be spoiled by the inroads of fungi. The best results were obtained with cultures made in Erlenmeyer flasks.

Cultures of Funaria protonema were kept in Erlenmeyer flasks from the first of Jan. to the 1st of May, four months, in perfectly sterile conditions, and in both light and darkness. Parallel with these was a culture started at the same time upon sterilized earth. The mode of growth of the protonema

on the earth agreed with that already described by Schimper,[1] two protonema axes generally being protuced from each spore and growing in opposite directions. There was almost a complete absence of any rhizoid production. Müller-Turgau[2] claims a quite abundant production of rhizoids by Funaria protonema. After growing for nine weeks the protonema had produced an abundance of buds, and rhizoids were then produced from the basiscopic cell of the bud.

Mention has already been made of the power of Barbula muralis protonema to separate into distinct cells, which are conidia-like and have the power of growing into new protonema. Sachs[3] speaks of this ability in regard to Funaria protonema and Schröder[4] states it as a general principle, that moss protonema when cultivated on too dry soil, break up into the separate cells, which are more resistant, and grown into new protonema under favorable conditions. In case of the leaf protonema of Barbula muralis I have shown that this manner of growth can not be due to dessication, since the culture was supplied with abundant moisture. In my cultures of Funaria protonema on earth this manner of growth was very marked. The cultures were supplied with a considerable amount of moisture, so that the separation into the individnal cells could hardly have been called forth by an insufficient amount. In the original culture the spores were sowed in the center of the Petri-dish, and after several weeks of growth only covered an area about 2 cm in diameter. After ten weeks nearly the whole Petri-dish was filled (6 cm in diameter) with a luxuriant growth of protonema, a large majority of which had grown from separated cells.

The cultures in the light on agar-agar produced no buds although they were exposed to sufficient illumination for four months. The control culture on earth had produced an abundance of buds after 9 weeks. The growth of the whole

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[1] l. c. Plate I.

[2] Arb. aus d. Bot. Inst. zu Würzburg, 1:480. 1874.

[3] Lehrbuch der Bot. 366. 1874.

[4] Unt. aus d. Bot. Inst. zu Tübingen, 2:15—21. 1886.

protonema was not as vigorous as in the control experiment. Figure 58 shows where the original spore cell has started to form a bud by the insertion of an oblique crosswall; further than this however no indication of bud formation was noted. The cultures in the dark produced protonema of considerable size and vigor, but the vigor of growth was markedly below the same cultures in the light. The protonema were perfectly free from chlorophyll and the considerable size attained shows that to a certain extent they are able to adapt themselves to a saprophytic mode of nourishment.

In the light the main protonema axes were directed parallel to the incident rays of light and grew either in or on the surface of the culture medium. The secondary branches grew erect from the prostrate axes, and directed themselves towards the light at an angle of about 45°, thus exhibiting a marked positive heliotropism. Sachs[1] has already referred to the so-called dorsiventrality of Funaria protonema. In the dark the main axes were without any definite direction, while the secondary branches grew more or less vertical but irregularly in all directions. Whether this vertical growth of the secondary branches in the dark is due to negative geotropism, I am not able as yet to state with certainty Some cultures which were grown in the dark in an inverted position showed that the protonema branches grew downward away from the culture medium. These experiments would tend to show that the vertical growth was not due to geotropic sensitiveness, but rather perhaps to negative hydrotropism as in the case of fungi. More experiments in this line are however necessary to make these conclusions certain.

It was thought that by using a culture medium with less peptone, that the protonema might be brought to a more vigorous development. A second series of experiments was curried out, in which the same culture medium as described above was used, except that only traces of peptone were added. Even in this medium the protonema did not grow

[1] Vorlesungen über Pflanzenphysiologie, p. 640. 1882.

with their normal vigor, either in light or darkness; the growth was however more luxuriant than in the cultures which were supplied with more peptone.

b. MARCHANTIA SPORES.

The work of Leitgeb upon the effect of light on the germination of liverwort spores has already been mentioned. Since he found that the spores were unable to germinate in complete darkness, a confirmation of his results at this point can not be without interest. A culture of Marchantia spores was kept in the dark for over two months without any signs of germination. At the end of this time they were placed in the light, and after a lapse of six days the majority of the spores had germinated. My experiments also confirmed his results in regard to the intensity of light necessary for germination. In weak light germination was retarded, and when growth did take place, the spores produced only a narrow filament with a small amount of chlorophyll. The filament did not attain any considerable size or form a germ disk.

As regards the part of the spectrum effective in producing germination, my experiments with Marchantia spores yielded the same results as for moss spores, and the same as has been found for fern spores by Borodin.[1] That is, the blue rays, the more strongly refrangible, have apparently the same effect as complete darkness. In the potassium dichromate light germination occurred after six or seven days, and with every evidence of as vigorous growth as under normal illumination.

The effect of temperature on the germination of Marchantia spores in the dark was also investigated. A series of cultures was made for 32°, 29°, 27°, 24° and 23° C and all placed in the dark. After two weeks time they were investigated and none of the spores had germinated. They were then placed in the light to see if they had remained capable of germination.

[1] C. c. p. 536.

In the cultures which had been kept at 29° and 32° C germination was retarded for a few days, but the the spores from the remainder of the cultures grew after the usual length of time.

The length of time required for germination, and the condition of the material rendered the preparation of sterile cultures impossible, so that the cultures with grape sugar were not very satisfactory. Although I did not succeed in bringing the spores to germinate under these conditions, they gave every indication of growth, and I attribute the failure to germinate only to the inroads of bacteria. In the dark in sugar solution, the spores increased to 3 or 4 times their original diameter and formed large starch masses the some as in the moss protonema.

Further than this they could not be brought, although repeated attempts were made, and when the cultures were placed in the light, even then germination did not proceed, showing that the spores had not remained capable of germination. If however the cultures could have been kept sterile, there was every indication that the spores would have germinated.

c. FERN SPORES.

A culture of the spores of Cerathopteris thalictroides was kept in the dark for three months without any signs of germination, while a sowing of the same spores germinated in the light after twelve days. Experiments with the spores Alcophila Loddigesii led to the same results. Thus for the species investigated, it can be stated as certain, that under ordinary conditions of nourishment and at a temperature of 19—21° C, the spores are not capable of germinating. The effect of a higher temperature was then tried for the spores of Cerathopteris, a culture of the spores being kept at 32° C in the dark. After a lapse of sixteen days the culture was examined and it was found that the spores had germinated abundantly. The form of growth was that of a cell filament, seven or eight cells long, the whole being about 2 mm in length. The basal cell always produced a rhizoid, and in

some cases the end cells of the prothallium divided also longitudinally. The comparative size and form of a prothallium grown under these conditions and one grown in light at the normal temperature is shown in Fig. 59 and 61. Experiments of a similar nature were also carried out for Alcophila Loddigesii. These experiments are interesting in that they show how Borodin and Schmidt, and Göppert and Schelting could have obtained such contradicting results.

d. EQUISETUM SPORES.

There are no contradicting views in regard to the germination of Equisetum spores, both investigations cited admitting and establishing the fact that germination occurred in perfect darkness. I have repeated these experiments for spores of Equisetum arvense, with the same result. It can then be stated with absolute certainty, that Equisetum spores are able to germinate under ordinary conditions of nourishment and at a temperature of 19—21° C, in darkness as well as in light. From the foregoing results it seems that light or organic nourishment is one of the necessary conditions for the germination of moss and liverwort spores, in order that chemical changes may take place which will bring the reserve food material into a condition in which it can be used in growth. For the ferns, these chemical processes may be initiated either by light or a sufficiently high temperature, while in the case of Equisetum these changes can occur at a much lower temperature in both light and darkness.

4. SUMMARY.

The more important results of the foregoing investigations may be stated as follows: —

1. Under ordinary conditions of temperature and inorganic nourishment, moss and liverwort spores are unable to germinate in the dark. Spores when subjected to only the more strongly refrangible rays of the spectrum, behave the same as in darkness.

2. Organic nourishment in the form of either peptone

or grape sugar will call forth the germination of moss spores in complete darkness. Moss protonema are able to attain a considerable size in the dark, by a saprophytic nourishment, although the vigor of growth is considerable below the normal.

3. Under ordinary conditions of temperature and inorganic nourishment, fern spores are unable to germinate in the dark. A higher temperature will however furnish conditions for the germination in complete darkness.

4. The spores of Equisetum germinate apparently as well in darkness as in light and at the ordinary room temperature of 19—21° C.

EXPLANATION OF PLATES.

MNIUM ROSTRATUM.

Fig. 1. Diagram of a leaf to show origin of rhizoids and buds. × 17.

„ 2. Cross section of a portion of a leaf showing the origin of a bud from a leaf cell, together with the previously produced rhizoid. × 195.

„ 3. Cross section of a portion of a leaf showing a bud at a more advanced stage. × 195.

„ 4. Portion of a leaf with rhizoids and a bud produced as a lateral branch of a rhizoid. × 80.

„ 5. Rhizoid (r) with protonema branches (p) which have been produced after exposure to light. × 195.

„ 6. Rhizoid (r) with protonema branches (p) showing the origin of a bud from a cell of the main rhizoid axis, and homologous with the lateral protonema branches. × 330.

„ 7. Same as 6, only the bud formation has occurred from one of the protonema branches direct. × 330.

FUNARIA HYGROMETRICA.

„ 8. Protonema and bud grown from the receptacle. × 195.

„ 9. Portion of a protonema as grown from a leaf in the dark. × 195.

„ 10. Paraphysis, showing the origin of a protonema from the basel cell. × 80.

„ 11. Proximal portion of the same on a larger scale. × 195.

„ 12. New shoot, rhizoids, and protonema growing from a defoliated stem. —

BRYUM CAPILLARE.

„ 13. Several cells from the leaf base showing the origin of protonema with the formation of a bud. × 195.

„ 14. Protonema and bud grown from the leaf. × 195.

„ 15. Bud and protonema grown from the leaf. × 80.

Fig. 16. Leaf base showing origin of protonema and the formation of a new leafy shoot. × 80.

„ 17. The basal half of a leaf, showing origin of protonema from both the proximal and the distal ends. × 80.

„ 18. Jip of a leaf showing protonema growing from the cut edge. × 80.

„ 19. Protonema and leaf cells from the preceding on a larger scale. × 195.

„ 20. Protonema originating directly from the defoliated stem. × 195.

„ 21. Paraphysis which has grown out into a protonema filament.

BRYUM ARGENTEUM.

„ 22. Leaf with protonema. × 80.

„ 23. A few of the marginal leaf cells showing the origin of the protonema. × 195.

„ 24. Leaf with protonema. × 80.

„ 25. A few of the basal cells showing the origin of the protonema. × 195.

„ 26. Portion of a defoliated stem showing the origin of protonema and the formation of buds. × 195.

BARBULA MURALIS.

„ 27. Basal portion of a leaf showing the origin of protonema. × 195.

„ 28. Portion of a leaf protonema showing a bud with rudimentary leaves. × 195.

„ 29. Protonema with rhizoids which have been produced in light in a ten weeks old culture. × 195.

ATRICHUM UNDULATUM.

„ 30. Protenema grown from the leaf and showing the aggregated branching so common in the leaf protomena of Atrichum. × 195.

„ 31. Portion of a protonema with bud. × 195.

„ 32. Cross section of a leaf showing the manner in which the protonema originate from the cells at the base of the outer lamellae. × 195.

POLYFRICHUM COMMUNE.

„ 33. Diagram of a leaf which has produced protonema and a leafy shoot. × 8.

„ 34. Portion of a cross section of a leaf showing the origin of a protonema from a cell at the base of a lamella. × 330.

„ 35. A bud produced direct by the end cell of the main protomena axis. × 330.

Fig. 36. A portion of a leaf protonema with aggregated branching. × 330.
„ 37. Protonema grown from leaf in the dark. × 195.
„ 38. „ „ „ „ „ „ light. × 195.
„ 39. Portion of a protonema, and a bud grown from a leaf in the dark. × 330.

BRACHYTHECIUM RUTABULUM.

„ 40. Cells from the leaf base, with bud and rhizo-protonema. × 330.

LEPTOBRYUM PYRIFORME.

„ 41. Cells of a rhizoid grown from a leaf in the dark. × 195.
„ 42. Continuation of the same filament after exposure to light showing a direct change to protonema. × 195.
„ 43. Protonema grown from a leaf axil, showing the formation of conidia-like cells by the lateral branches. × 195.
„ 44. Two of the separated protonema cells. × 330.
„ 45. Germination of two of these cells. × 195.
„ 46. Early stage in the formation of an axillary brood-body. × 195.
„ 47. Mature brood-body with is stalk. × 195.
„ 48. Protonema grown from the stem, and showing two lateral branches often coming from a single cell of the main axis. × 80.
„ 49. Protonema with a brood-body, similar to those produced in the leaf axil. × 195.

PHASCUM CUSPIDATUM.

„ 50. Leaf showing origin of protonema and the formation of a new leafy shoot. × 40.
„ 51. Cross section of a leaf showing the origin of a protonema from a cell of the ventral surface. × 330.
„ 52. Portion of a protonema with a bud. (d) Cells just above the costa. × 330.

FISSIDENS BRYOIDES.

„ 53. Bud grown from the leaf axil of a defoliated stem without the intervention of a protonema. × 330.
„ 54. Rhizoid grown from a defoliated stem in the dark with perpendicular cross walls produced by intercallary growth. × 195.

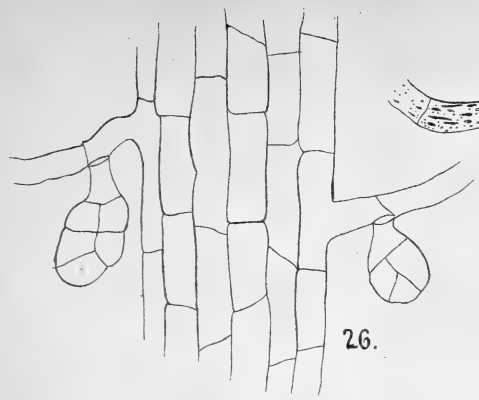
„ 55. Various stages of Funaria spores germinated in the light (a). After being in culture for 3 days. × 330.
„ 56. Spores of Funaria which germinated in the dark in a sugar solution. a & b. After being in culture for 3 days. × 330.

Fig. 57 & 58. Protonema of Funaria grown in dark; eight days on peptone, sugar and agar-agar. × 330.

„ 59. Prothallium of Cerathopteris thalyctrioides grown in the dark at 32° C. In culture 16 days. × 330.

„ 60. Apical portion of a prothallium of the same species showing longitudinal a well as transverse divisions. × 330.

„ 61. Germinating spore of the same age as in 59, only grown in light at a temperature of 19°—21° C. × 330.



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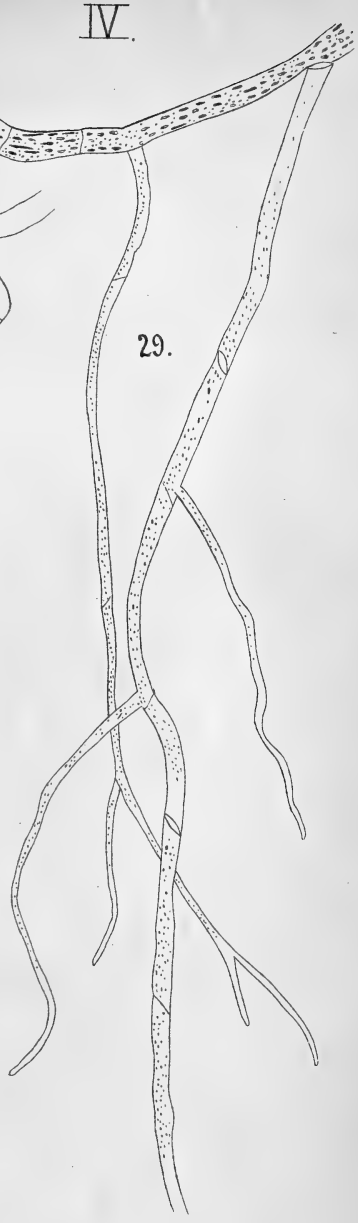

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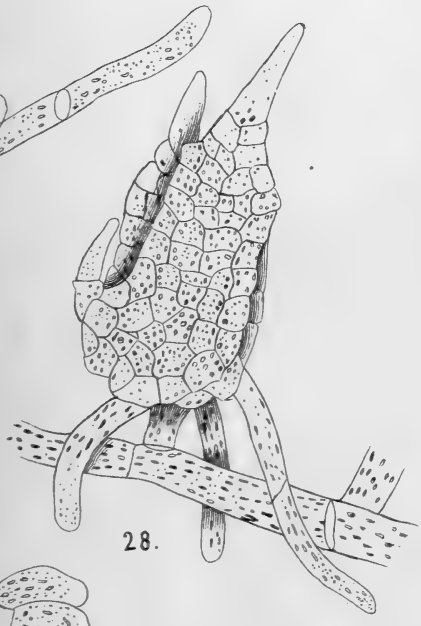

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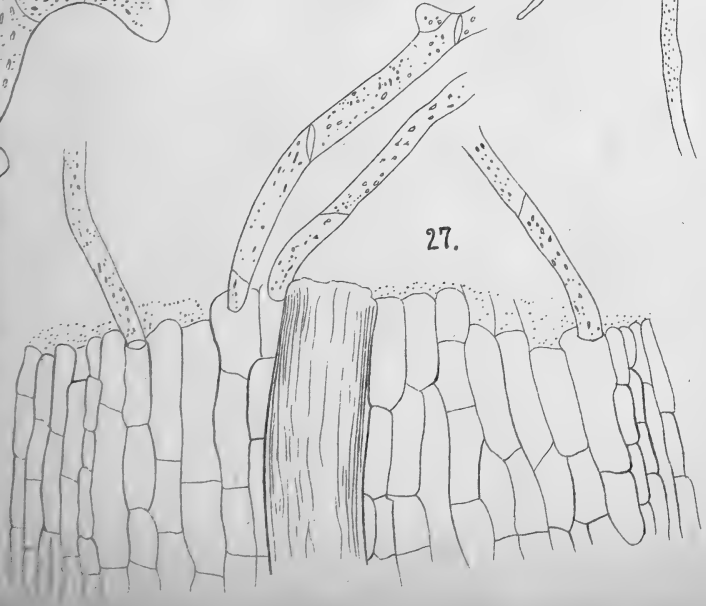

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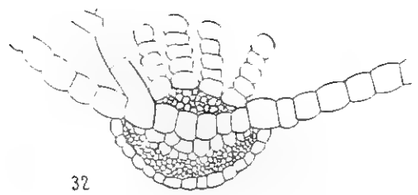

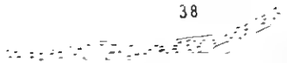

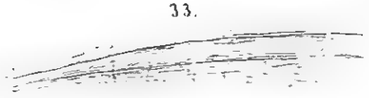

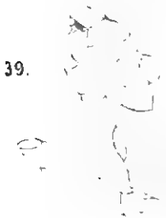

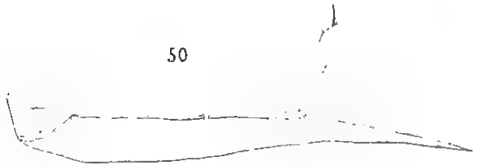

Lebenslauf.

Ich, Fred De Forest Heald, Anhänger des presbyterianischen Glaubens, wurde am 23. Juli 1872 zu Midland City im Staate Michigan, V. S. A., als Sohn von Henry F. und Hettie B. Heald geboren. Den ersten Unterricht erhielt ich von meinen Eltern. Ich besuchte dann die Bürgerschule meiner Vaterstadt, um später die besondere Vorbereitung zum Universitätsstudium auf der Vorbereitungsschule der Universität zu Vermillion, Süd-Dakota, vorzunehmen.

Im Jahre 1891 an der Wisconsin Universität zu Madison, Wisconsin immatrikulirt, reichte ich im Jahre 1894 bei der philosophischen Facultät eine Arbeit ein: „Concerning the Comparative Histology of Pulvini and the Resulting Photeolic Movements", durch welche ich mir die Würde eines „Bachelor of Science" mit besonderer Auszeichnung erwarb. An derselben Universität wurde ich dann sofort zum „Fellow" in der Botanik ernannt, welche Stellung ich während zweier Jahre bekleidete. Während dieser Zeit ertheilte ich Unterricht in Biologie und setzte dabei mein wissenschaftliches Studium fort. Am Ende des zweiten Jahres überreichte ich der philosophischen Facultät eine Original-Abhandlung: „On the Joxic Effect of Dilute Solutions of Acids and Salts upon Plants", und nachdem ich die vorgeschriebene Prüfung bestanden hatte, erhielt ich die Würde eines „Master of Science." Während dieser Zeit beschäftigte ich mich mit einer Revision von Prof. Chas. R. Barne's „Keys to the Genera and Species of North American Mosses", welche am Anfange des laufenden Jahres erschien.

Im Herbst 1896 verliess ich die Wisconsin Universität, um meine Studien an der Universität Leipzig fortzusetzen. Im Oktober wurde ich daselbst immatrikulirt und besuchte die Vorlesungen der folgenden Herren Professoren: A. Fischer, Leuckart, Pfeffer und Wiedemann. Unter der Leitung des Herrn Geheimrath Professor Doctor Pfeffer habe ich die in dieser Dissertation niedergelegten Untersuchungen ausgeführt.