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RESPONSES OF MARCHANTIA POLYMORPHA TO NUTRIENT SUPPLY AND PHOTOPERIOD[1, 2]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 515

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(WITH FOURTEEN FIGURES)

Introduction

As experimental material in studies on plant nutrition, *Marchantia polymorpha* L. has been used for many years. Various substrates have been used, including soil (5, 29, 31, 33), agar with various nutrients (6, 8, 10, 23, 34), blotting or filter paper (5, 6, 29), sand (29), cinders (38), peat (29, 31), and nutrient solutions (1, 5, 9, 10, 39). These investigations have utilized gemmae in the main and include studies of dorsiventrality (1, 5, 8, 29, 31, 39), effect of the concentration and composition of nutrient solution (1, 2, 10, 11, 23, 33), effect of temperature variations (1, 5, 9, 10, 11), intensity and quality of light (5, 6, 8, 9, 10, 11, 23, 34, 39), and photoperiod (5, 38). Work on seed plants (17, 28) has indicated a possible interrelationship between mineral nutrition and photoperiod. The present studies deal principally with similar relationships in *Marchantia*, with particular reference to the development of the gametophyte and gametangiophores.

Marchantia grows readily on moist soil, but our attempts to grow it in sand culture have been more or less unsuccessful because of the tendency of the surface of the sand to become dry, and consequently the plants do not obtain sufficient moisture. When a system of continuous irrigation in sand was used, salts tended to accumulate on the surface of the sand, resulting in injury to the plants. Most of these difficulties were avoided by the use of methods subsequently described in this paper. Plants were grown on a wide variety of nutrient solutions, under various conditions of photoperiod. Records were made in each case of changes in the gross appearance of the plants, increase in total area, formation of gemmae cups and gametangiophores, anatomical variations, and the accumulation of dry weight.

General methods

The strains of *Marchantia polymorpha* used in these experiments produce (as usually grown) antheridiophores in late February and early March and archegoniophores a few weeks later. Gemmae cups are produced abundantly in autumn

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and winter. The experiments described here were conducted in the greenhouses at the University of Chicago during the late spring, summer, and fall of 1939 and the spring of 1940.

Sand culture having proved unsatisfactory, floats of balsa wood were devised to support the thallus and to keep it in contact with the surface of the culture solu-

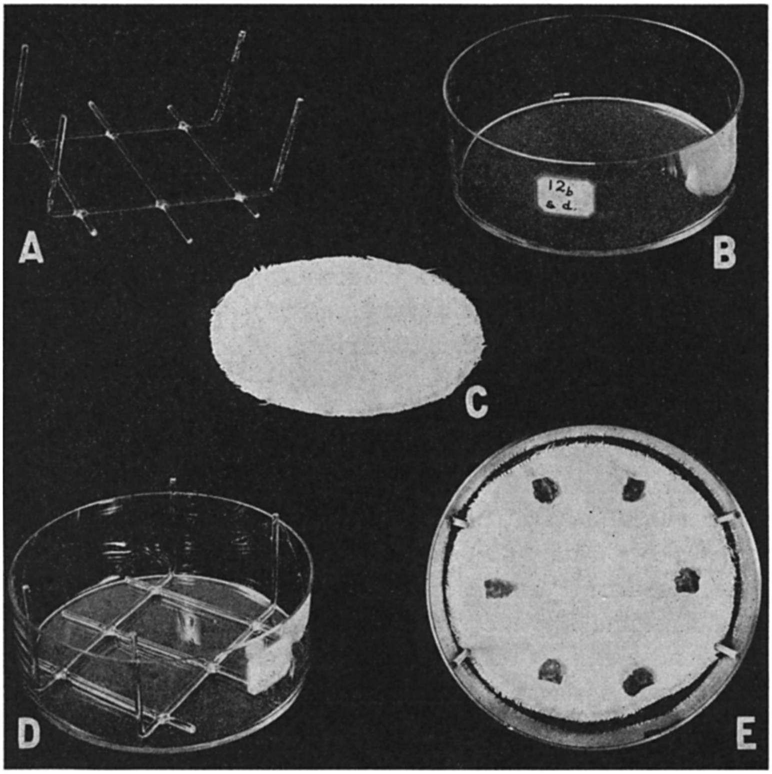

FIG. 1.—Apparatus used in growing *Marchantia: A*, glass rack or framework welded from pieces of glass rod, 5 mm. in diameter; *B*, half a glass moist chamber, approximately 9×3 inches; *C*, glass cloth; *D*, glass rack in moist chamber; *E*, complete assembly with six cuttings.

tion. This method was abandoned because the floats tended to become water logged, even though impregnated with paraffin.

After several preliminary trials, it was found that *Marchantia* would grow well if placed upon a coarse grade of glass cloth in contact with nutrient solution. The cloth served as a wick and was moist throughout its area if kept in contact with the solution at one or two points. The setup used is illustrated in figure 1. Half of a moist chamber was placed on a greenhouse bench with the open face upward. Glass rods were welded together to form a circular framework which would just fit into the chamber, and over this framework a circular piece of glass cloth was so placed that it was approximately $\frac{1}{4}$ inch above the bottom of the chamber.

Sufficient culture solution was added to come into contact with the lower surface of the cloth. Extensions of the two longer rods of the framework were bent upward to serve as handles in facilitating removal from the moist chamber. Before use in establishing a culture in any experiment, the entire setup was thoroughly cleansed by treatment for 24 hours in cleaning solution and then thoroughly washed in tap water, followed by distilled water. All nutrient solutions were changed every day or every other day, depending upon the temperature and the amount of evaporation. Every second day the framework and glass cloth were removed and rinsed thoroughly in distilled water, and fresh nutrient solution was again placed immediately in the moist chamber.

Using this method, an attempt was made to grow *Marchantia* on a solution which had an osmotic concentration of approximately 0.79 atmospheres. At full strength this solution consisted of 12 cc. of 0.5*M* $Ca(NO_3)_2$, 9 cc. of 0.5*M* KH_2PO_4, 9 cc. of 0.5*M* $MgSO_4$, and 1 p.p.m. of some of the microelements—except $FeSO_4$, which was supplied at 0.1 p.p.m. (**16**). The plants grew fairly well in this solution when compared with similar plants grown on soil. There was, however, some evidence of salt excess in the nutrient cultures; consequently, several experiments were conducted in which the growth of *Marchantia* on the full strength solution was compared with its growth on dilutions of one-fifth and one-tenth the original concentration. Control plants were grown on soil, tap water, and distilled water. Responses to the one-tenth dilution were similar to the one-fifth dilution. Because of the growth responses, production of gametangiophores and gemmae cups, and area of thallus developed, the concentration of solution used as a basis in making up the nutrient combinations in all subsequent experiments was comparable to that of the one-fifth dilution of the solution originally employed. It is not known whether the greater growth was owing to a lower total osmotic concentration or to a lower concentration of certain of the individual inorganic salts. In all subsequent work the solutions had an osmotic concentration of approximately 0.285 atmospheres.

A range of nutrient solutions was used according to the method reported by C. L. Hamner (**16**). Six stock solutions were prepared with molal concentrations as given in table 1. Each of three of these contained only K, Ca, or Mg as the cation, but all contained the three anions NO_3, PO_4, or SO_4. Each of the other three contained only NO_3, PO_4, or SO_4 as the anion, but had the cations K, Ca, and Mg. This was accomplished by the use of nine salts, as indicated in table 1. By the use of these solutions, and by effecting various combinations of them on the basis of sixths, it was possible to arrange a triangle of twenty-eight different solutions with special reference to the cations present and an additional twenty-eight with particular reference to the anions. The combinations composing each triangle were placed in order on the basis of their relative composition and numbered (fig. 2). The twenty cultures on short photoperiod were supplied with solutions such as occurred only in the central portion of the triangle, ten representing the cations and ten the anions. The pH of all stock solutions (table 1) and those supplied the plants (fig. 2) was between 5.0 and 6.0. All solutions became neutral or slightly alkaline after being in the moist chambers in contact with the growing plants for a day, except the solutions lacking in nitrogen, which changed very little.

The conditions for long photoperiod were obtained by means of 200-watt Mazda lamps with reflectors suspended about 3 feet above the greenhouse bench. These lamps burned from sundown until 2:00 A.M. each night, supplying a maximum illumination of approximately 100 foot-candles at the surface of the plant.

TABLE 1

CONCENTRATIONS OF SALTS IN THE 6 STOCK NUTRIENT SOLUTIONS USED IN MAKING UP 56 OTHER COMBINATIONS. TO EACH STOCK SOLUTION WAS ADDED 0.2 P.P.M. OF $MnSO_4$, $ZnCl_2$, AND $Na_2B_4O_7$, AND ALSO 0.02 P.P.M. OF $FeSO_4$

Salt	Molal concentrations of individual stock solutions					
	K	Ca	Mg	NO_3	PO_4	SO_4
KNO_3	0.0024			0.0009		
KH_2PO_4	0.0009				0.0009	
K_2SO_4	0.0009					0.00045
$Ca(NO_3)_2$		0.0012		0.0012		
$Ca(H_2PO_4)_2$		0.00045			0.0012	
$CaSO_4$		0.0009				0.0012
$Mg(NO_3)_2$			0.0012	0.0009		
$MgHPO_4$			0.0009		0.0009	
$MgSO_4$			0.0009			0.0009

The short photoperiod was obtained by building a framework over the bench and covering it completely with black cloth at 5:00 P.M. every evening. The black cloth was removed at 8:00 A.M. each morning. Thus the plants exposed to long photoperiod received approximately 18–20 hours of light out of each 24-hour period, and plants exposed to short photoperiod received 9 hours of light.

All the plants were propagated vegetatively from a single male plant and a female plant. They were grown in the greenhouse on a one-fifth dilution of a three salt solution (**16**) and furnished the stock from which the plants used for experimentation were taken. At the beginning of any particular experiment, a small portion from the tip of a branch of the stock was taken. Six of these cuttings were selected for uniformity and arranged equidistantly on the glass cloth (fig. 1*E*). The growing point of each cutting was directed toward the center of the dish. At the time of making the cuttings, the total area of each particular group of six was determined by means of an area photometer (**24**). Several representative groups of six cuttings designated as initial controls were also set aside for fresh and dry weight determinations, and other representative cuttings were fixed for subsequent sectioning in a formalin-acetic-alcohol solution consisting of 67 cc. forma-

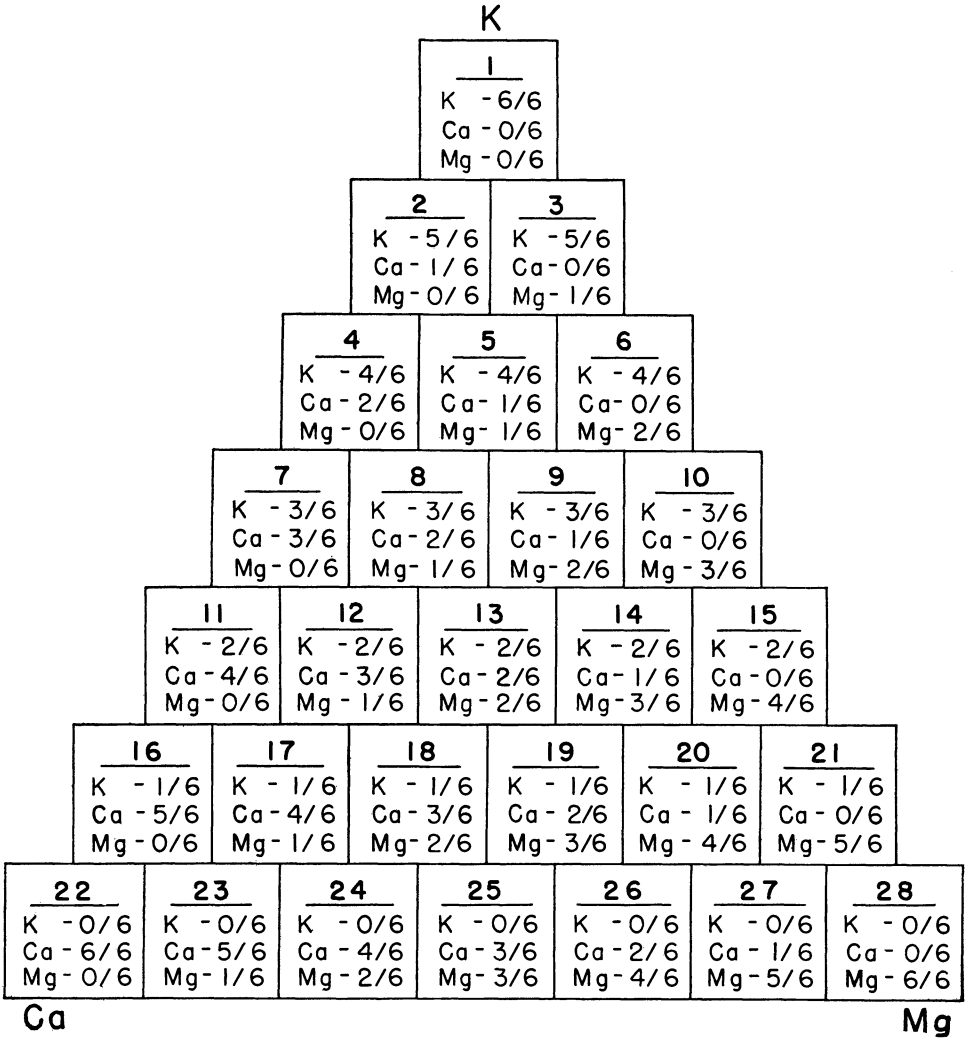

FIG. 2.—Triangle showing method of combination of stock solutions on the basis of sixths, to secure a range of 28 solutions of varying cations. Each apical position lacks two cations; each side of the triangle lacks one; and the center is supplied with all three in varying proportions. A triangle for varying the combination of anions could be effected in a similar manner.

lin, 23 cc. glacial acetic acid, 500 cc. of 95 per cent alcohol, and water sufficient to make 1 liter.

At the completion of an experiment, the plants were carefully loosened from the glass cloth by means of a sharp spatula, each group was photographed, and the total area was determined. Careful notes were made as to the number of gemmae cups, the number of antheridiophores or archegoniophores, and the general appearance of the plants. The fresh weight of the plants was then determined, and one plant selected for fixation in F.A.A. and subsequent sectioning. The fresh weight of the remaining five plants was determined. They were then dried at 102° C. On the basis of the percentage dry weight of five plants, and the fresh weight of the six plants, the total dry weight of six plants was calculated.

Investigation

Details of specific experiments

Experiment 8.—On March 14, 1940, fifty-six cultures were placed under conditions of long photoperiod and twenty under short photoperiod. The six cuttings in each culture totaled 15 sq. cm. in area. In any set of six plants there were approximately 16–18 growing points. Four more cultures served as initial controls. The fresh and dry weights of each lot varied very little. The average dry weight was 60 mg. The character of growth, development, and final results obtained under conditions of short photoperiod are described later in a special section. The growth and general appearance of the plants subjected to conditions of long photoperiod at progressive dates were as follows.

On March 22, 8 days after beginning the experiment, progressive death was noted in certain of the cultures in the cation triangle on long photoperiod. The growing points of the plants supplied with nutrient solutions lacking in calcium were dead, and necrosis in the wings of the thallus followed. The most rapid development of this symptom occurred in nutrient solution 28. The other solutions lacking calcium ranged in this order of toxicity: 21, 15, 10, 6, 3, and 1. While all these solutions are lacking in calcium, they contain various proportions of magnesium and potassium. Plants on the solutions relatively high in magnesium and low in potassium seemed to develop the calcium deficiency symptoms more rapidly than did those on solutions high in potassium and low in magnesium. The first evidence of calcium deficiency was darkening of the growing point, which later became translucent, then very black, and finally died completely. Fourteen days after the start of the experiment, or March 28, the calcium deficiency symptoms were much more apparent. On April 2, growing points of all the plants supplied with a nutrient solution lacking calcium were dead, and portions of the thallus were disintegrated. On April 16 there was some regeneration in the plants growing on solutions lacking in calcium. This was particularly true with the plants on solutions 1, 3, 6, and 10. Slight regeneration was apparent in the plants on solution 15, and those plants on solutions 21 and 28 were apparently dead.

There were no particular symptoms of magnesium deficiency on April 2, except that the plants on solutions lacking magnesium grew less than some of the others.

The greatest growth in the cation triangle was made by plants receiving solutions 8, 11, 12, 13, 17, and 18. The plants supplied with nutrient solutions lacking potassium did not grow so well, and the older portions of the thallus of such plants had died. This portion of the thallus was often straw colored rather than bright green. On April 16, approximately 50 per cent of the base of each plant supplied with solutions lacking in potassium was dead. There was very little evidence of magnesium deficiency in any of the plants at this time, except perhaps that the plants on solutions lacking magnesium did not grow quite so rapidly. No evidence of potassium or calcium excess symptoms was recorded. There may have been some effect of excess in magnesium, as evidenced by the fact that plants on solution 28 were apparently dead early in the course of the experiment. Plants on solutions 21 and 15 also appeared dead by the time of harvest. The fact that solutions 21 and 15 were not so favorable for growth as were solutions 1 and 3 may have been owing to the presence of a higher concentration of magnesium in solutions 21 and 28 than in solutions 1 and 3, or possibly to the lower concentration of potassium in the former as compared with the latter. All these solutions, of course, contained no calcium.

By March 28, certain types of growth responses were evident in the plants of the anion triangle exposed to long photoperiod. Those plants growing on solutions lacking phosphorus were somewhat greener than the others, and certain of them developed a green-black color in the midrib region. By April 2, all the plants on nutrients lacking phosphorus had black midribs. At this time the greatest growth in the anion triangle was on solutions 31, 33, 34, 36, 37, and 38. Up to this time absence of phosphorus had not resulted in appreciable decrease in the rate of growth, if at all. On April 16, the plants on solutions lacking phosphorus all exhibited extensive growth and were of darker green color than plants on any of the other solutions. Particularly the regions near the tips of the thallus were dark green, the midrib was black or very deep red, and the bases of the plants were tan. On this date, those plants growing on solutions lacking nitrate were stunted, producing much less growth than those on other solutions. They had a red coloration which at first was ascribed to anthocyanin in the scales on the ventral surface of the thallus. This red color and the color of the plants lacking in phosphorus will be discussed in the anatomical section. The margins of the thalli possessed very little chlorophyll and were almost white. Those plants growing on solutions lacking in sulphate produced slightly less growth than those on some of the other solutions. There was some evidence of phosphate excess in plants growing on solutions 50, 44, 51, 39, 45, and 52. The margins of these plants were necrotic and subsequently tended to dry up, as if burned. This symptom, however, was not very marked at any time.

All plants were harvested on April 16 (figs. 3, 4, 7, 8). The records of the num-

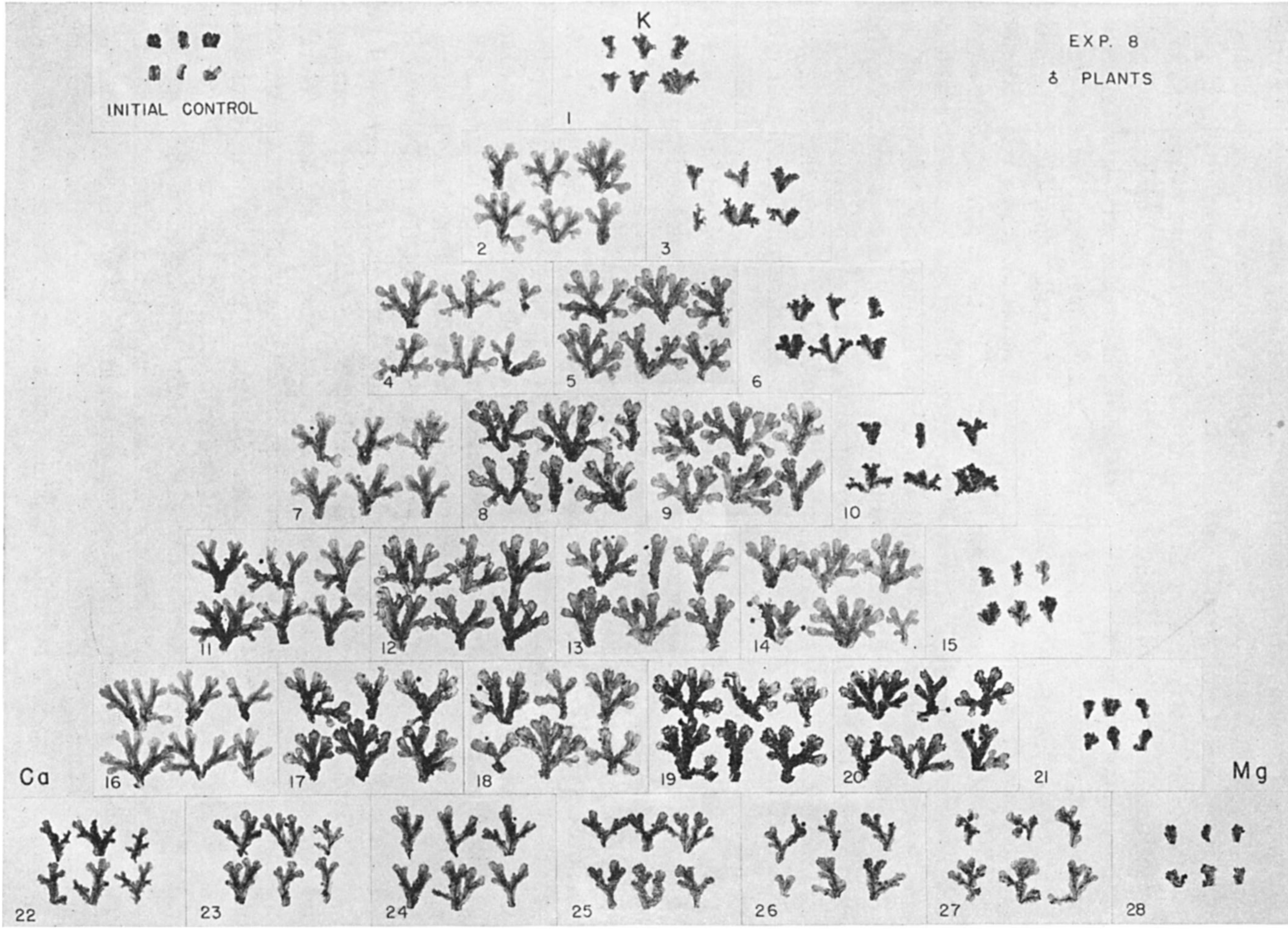

FIG. 3.—Cation triangle of experiment 8, April, 1940; antheridial plants on long photoperiod.

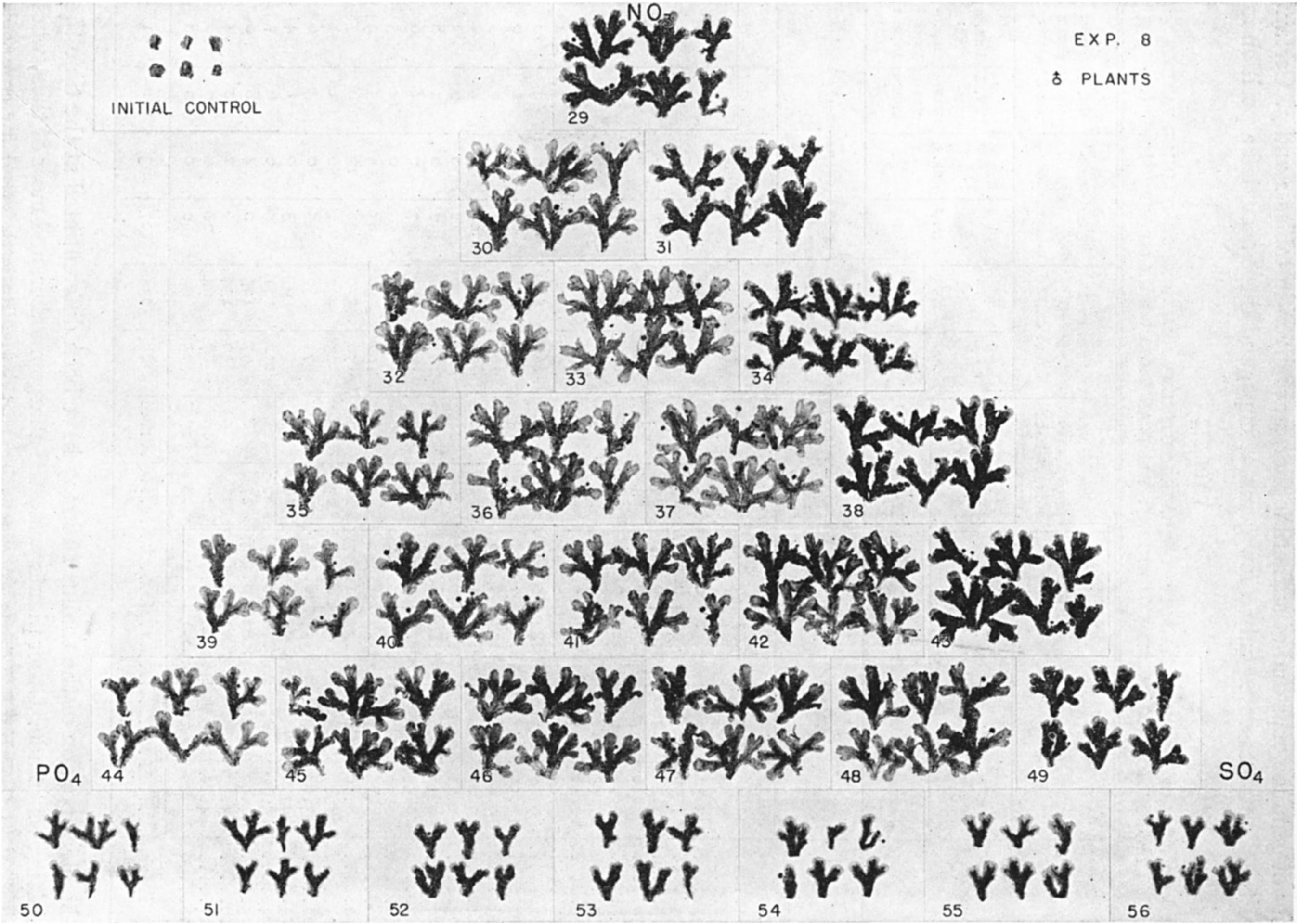

FIG. 4.—Anion triangle of experiment 8, April, 1940; antheridial plants on long photoperiod

ber of gemmae cups and of antheridiophores are given in tables 2 and 3. Certain of the plants possessed only a few gemmae cups, and these occupied a position in

TABLE 2

GROWTH OF MARCHANTIA POLYMORPHA ON NUTRIENT SOLUTIONS CONTAINING VARIOUS PROPORTIONS OF CATIONS OR ANIONS. ALL PLANTS GROWN ON LONG PHOTOPERIOD

Position in triangle	Weight of 6 plants (gm.)				Rank of various solutions in each triangle on basis of dry weight of 6 plants		Area of 6 plants (sq. cm.)		No. of gemmae cups on 6 plants		No. of gametangiophores on 6 plants	
	Exp. 8		Exp. 9				Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8 Antheridiophores	Exp. 9 Archegoniophores
	Fresh	Dry	Fresh	Dry	Exp. 8	Exp. 9						
	Cations K, Ca, and Mg varied proportionately on basis of sixths (cf. table 1) Anions present in constant relative proportion											
1......	2.88	0.257	2.72	0.095	25	28	47	34	3	0	0	0
2......	8.69	0.587	6.32	0.356	14	21	158	103	81	0	4	3
3......	3.32	0.283	3.51	0.302	24	22	50	60	9	0	1	4
4......	7.06	0.581	7.23	0.523	15	14	145	129	50	6	15	1
5......	13.36	0.780	19.94	0.917	4	10	186	324	126	66	16	1
6......	3.40	0.309	4.38	0.272	23	25	55	70	0	0	4	1
7......	7.76	0.601	7.23	0.493	13	17	137	122	27	1	9	4
8......	12.93	0.848	21.38	1.155	1	4	201	375	126	9	7	0
9......	12.42	0.737	21.27	1.046	7	8	230	381	104	21	8	2
10......	3.49	0.317	6.95	0.487	22	18	65	105	2	7	3	4
11......	9.07	0.746	6.82	0.607	6	13	161	134	27	0	6	10
12......	11.55	0.813	18.62	1.123	3	5	219	315	86	3	1	0
13......	10.48	0.678	16.53	0.980	10	9	184	286	97	6	8	5
14......	10.21	0.667	23.54	1.314	11	2	200	442	83	41	4	2
15......	1.71	0.153	3.38	0.273	26	24	40	56	2	0	0	2
16......	8.15	0.703	7.86	0.619	9	12	167	136	2	0	1	8
17......	10.45	0.825	16.18	1.060	2	7	197	283	47	0	11	6
18......	10.46	0.718	18.75	1.123	8	6	178	374	57	0	12	5
19......	11.16	0.763	27.33	1.741	5	1	192	496	85	14	5	4
20......	8.76	0.654	21.33	1.297	12	3	170	370	58	13	6	7
21......	1.27	0.132	2.51	0.200	27	26	25	37	1	0	1	1
22......	3.68	0.337	2.54	0.283	20	23	73	36	5	0	3	6
23......	4.70	0.403	5.09	0.473	18	19	95	87	3	0	1	4
24......	5.76	0.477	7.58	0.697	16	11	112	141	9	0	6	7
25......	4.02	0.404	5.83	0.494	17	16	97	105	0	4	8	0
26......	3.49	0.328	7.27	0.518	21	15	80	134	9	2	11	4
27......	4.18	0.339	5.00	0.423	19	20	87	94	28	0	10	0
28......	1.00	0.102	1.94	0.164	28	27	25	25	0	0	0	0
Totals......	....	....	....	....	......	......	3576	5254	1127	193	161	91

the older portion of the thallus and may have been differentiated at the time the cutting was made. Other plants possessed a large number, and most of these were present in the younger areas. A discussion of the results is deferred for comparison with the results of experiment 9.

EXPERIMENT 9.—On April 26, an experiment using cuttings from archegonial plants as described for experiment 8 was begun.

TABLE 2—*Continued*

Position in triangle	Weight of 6 plants (gm.)				Rank of various solutions in each triangle on basis of dry weight of 6 plants		Area of 6 plants (sq. cm.)		No. of gemmae cups on 6 plants		No. of gametangiophores on 6 plants	
	Exp. 8		Exp. 9									
	Fresh	Dry	Fresh	Dry	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8 Antheridiophores	Exp. 9 Archegoniophores
	Anions NO_3, PO_4, and SO_4 varied proportionately on basis of sixths (cf. table 1) Cations present in constant relative proportion											
29......	10.48	0.755	12.26	0.720	8	14	196	202	96	89	21	4
30......	12.52	0.795	33.66	1.861	6	1	194	532	52	30	13	1
31......	9.91	0.745	12.79	0.794	11	13	183	234	96	97	16	4
32......	12.59	0.806	21.62	1.142	5	5	210	370	62	22	8	3
33......	14.38	1.012	29.06	1.755	1	2	230	507	69	18	11	0
34......	10.10	0.727	14.21	0.926	15	9	205	252	105	50	31	16
35......	8.86	0.577	8.83	0.529	19	20	170	167	11	1	2	4
36......	12.80	0.837	15.63	0.880	4	10	236	284	62	0	11	5
37......	11.15	0.919	21.91	1.258	2	4	234	401	64	10	19	2
38......	9.56	0.738	16.17	1.069	13	7	197	280	86	6	19	6
39......	10.94	0.686	14.50	0.874	16	11	142	250	20	1	10	5
40......	8.78	0.753	13.29	0.811	9	12	175	264	47	0	14	5
41......	8.55	0.610	17.61	1.090	18	6	173	325	29	0	8	8
42......	11.72	0.745	22.36	1.353	10	3	244	405	75	0	5	10
43......	11.13	0.851	8.72	0.523	3	21	220	172	84	48	30	0
44......	7.48	0.513	11.30	0.678	21	16	150	174	22	0	16	10
45......	9.50	0.740	8.08	0.595	12	19	208	165	25	0	7	8
46......	9.28	0.629	9.96	0.689	17	15	204	188	14	0	5	11
47......	10.31	0.734	9.33	0.596	14	18	211	189	34	1	6	19
48......	10.32	0.783	14.57	1.039	7	8	215	288	64	0	11	10
49......	7.21	0.559	9.07	0.601	20	17	158	174	44	2	18	11
50......	3.23	0.265	3.05	0.220	25	24	80	47	4	0	9	3
51......	3.41	0.230	3.35	0.219	26	25	88	50	2	0	7	14
52......	2.93	0.206	2.57	0.181	28	27	75	42	2	0	5	6
53......	3.09	0.222	2.38	0.172	27	28	80	44	2	0	4	2
54......	3.46	0.286	2.57	0.204	24	26	77	48	1	0	9	7
55......	3.98	0.343	2.95	0.234	22	23	90	71	1	0	15	4
56......	3.63	0.331	3.18	0.250	23	22	80	67	0	0	15	5
Totals.	....	....	....	....	....	......	4725	6192	1173	375	345	183

On May 19, the plants under conditions of long photoperiod and supplied with solution 1 were watery in appearance, and some of them became infected with molds. Most of these plants were practically dead at this time. These symptoms may be attributable to the presence of a high concentration of potassium or to the combined absence of magnesium and calcium. On this same date, the plants supplied with solution 22 were much smaller than most of the other plants and were dark green in color, streaked with black or deep red along the midribs. The older portion of the thallus was streaked with yellow-green. The plants supplied with solution 28 had grown very little up to this date, were black in color, and all were dead. Plants supplied with solution 21 had some of the symptoms apparent in those plants supplied with solution 28, although they had not progressed so far. The plants supplied with solution 15 showed some symptoms similar to those developed in 21 and 28. Apparently, if calcium is lacking, a high concentration of magnesium—together with a low concentration of potassium—is definitely toxic. Those plants supplied with solutions lacking in potassium (that is, supplied with solutions 22 to 28, inclusive) developed characteristic symptoms. The margins of the older portion of the thallus turned light tan to brown, finally becoming watery in appearance, and ultimately the margins became dry. Progressive stages of this symptom were characterized by concentric rings, each ring marked by a slightly darker brown color, particularly noticeable in the notches between the branches of the thallus. Those plants supplied with solutions lacking in calcium (solutions 1, 3, 6, 10, 15, 21, and 28) developed more or less characteristic symptoms, as described for experiment 8. In this particular group, plants on solution 10 grew the most. There was some evidence of regeneration of the thallus.

Those plants supplied with solutions lacking in magnesium (solutions 1, 2, 4, 7, 11, 16, and 22) did not develop any characteristic symptom. Plants supplied with solution 22 produced very little growth, and there was some burning in the margins of the plants supplied with solutions 2, 4, 7, 11, and 16. The thallus of plants supplied with solution 1 had extensive burning at the margins and grew very slightly. On this date all the plants in the central portion of the triangle were about equal in gross appearance.

On May 19, the plants supplied with solutions of the anion triangle were also carefully examined. Those supplied with solutions lacking in nitrates (solutions 50 to 56, inclusive) were all small and had produced only enough growth approximately to double the original area of the cuttings. All these plants were pale yellow-green in color, and the scales on the ventral surface were bright red. This color could be seen showing through the more or less transparent thallus. The tips of the thalli were light green, and branches were somewhat narrow at the tip. Most extensive growth was produced by those plants supplied with solution 56, and decreased growth was apparent in order of solution number, down to solution 50. It was noted, in caring for these plants lacking in nitrates, that rhizoids developed in abundance very early after starting the experiment. Those plants supplied with solutions lacking in phosphorus (solutions 29, 31, 34, 38, 43, 49, and 56) were dark green and produced an abundance of dark-colored rhizoids. The midribs were almost black, with a slight reddish hue. Except for those plants supplied with solution 56, which were much smaller than the others, all the plants in this group were practically alike in size and produced about as much growth as did the plants in other positions in the triangle, with the exception of those mentioned immediately below. Those plants supplied with solutions 30, 33, 37, 42, and 48 appeared at this time to have made much more growth than the other plants in the triangle. The only characteristic of this particular group which would account for their similarity in gross appearance at this time is the fact that all these solutions contained one-sixth of the PO_4 solution. This might indicate that maximum growth of *Marchantia* is associated with low concentration of phosphorus, which is at variance with the results attained with thalli grown from gemmae (**33**). A plant growing on solution 30 increased in area from 2.5 to 160 sq. cm. in 32 days. All plants in this experiment were harvested on May 27 (figs. 5, 6, 7, 8).

Discussion of experiments 8 and 9

The results of fresh and dry weight determinations of experiments 8 and 9 are recorded in tables 2 and 3. It is apparent that there were great differences in the amount of dry weight produced by various groups of plants supplied with the different solutions. On the basis of these dry weights, the rank of the various solutions was determined. Thus the solution with rank no. 1 produced the greatest amount of dry weight, and the solution with rank no. 28 produced the least, the other solutions ranking intermediately. The results of the counts on gemmae cup formation, antheridiophore and archegoniophore formation, and the area in square centimeters are recorded in tables 2 and 3.

The rate of accumulation of dry weight by *Marchantia* is markedly associated with the variations in nutrient solutions. In the cation triangle of both experiments 8 and 9, greatest growth was produced on the solutions in the central portion of the triangles. There was little change in the comparative rank of any given solution, regardless of the system used for calculation—whether on the basis of fresh weight or dry weight and of five or six plants. The responses of the antheridial plants are slightly different from those of the archegonial plants, but the correspondence is close considering the fact that the two experiments were carried out at different seasons of the year and under different conditions of intensity of daylight and average temperature.

On those solutions lacking in calcium the least growth was produced, and of this group the most growth was on solution 10, which contains about equal amounts of magnesium and potassium. As a group, it may be noted also that on those solutions lacking in potassium relatively little growth took place. A lack of magnesium did not affect the growth as much as the other absences, particularly in those solutions in which the calcium content was relatively high and the potassium content relatively low; for example, solutions 11 and 16.

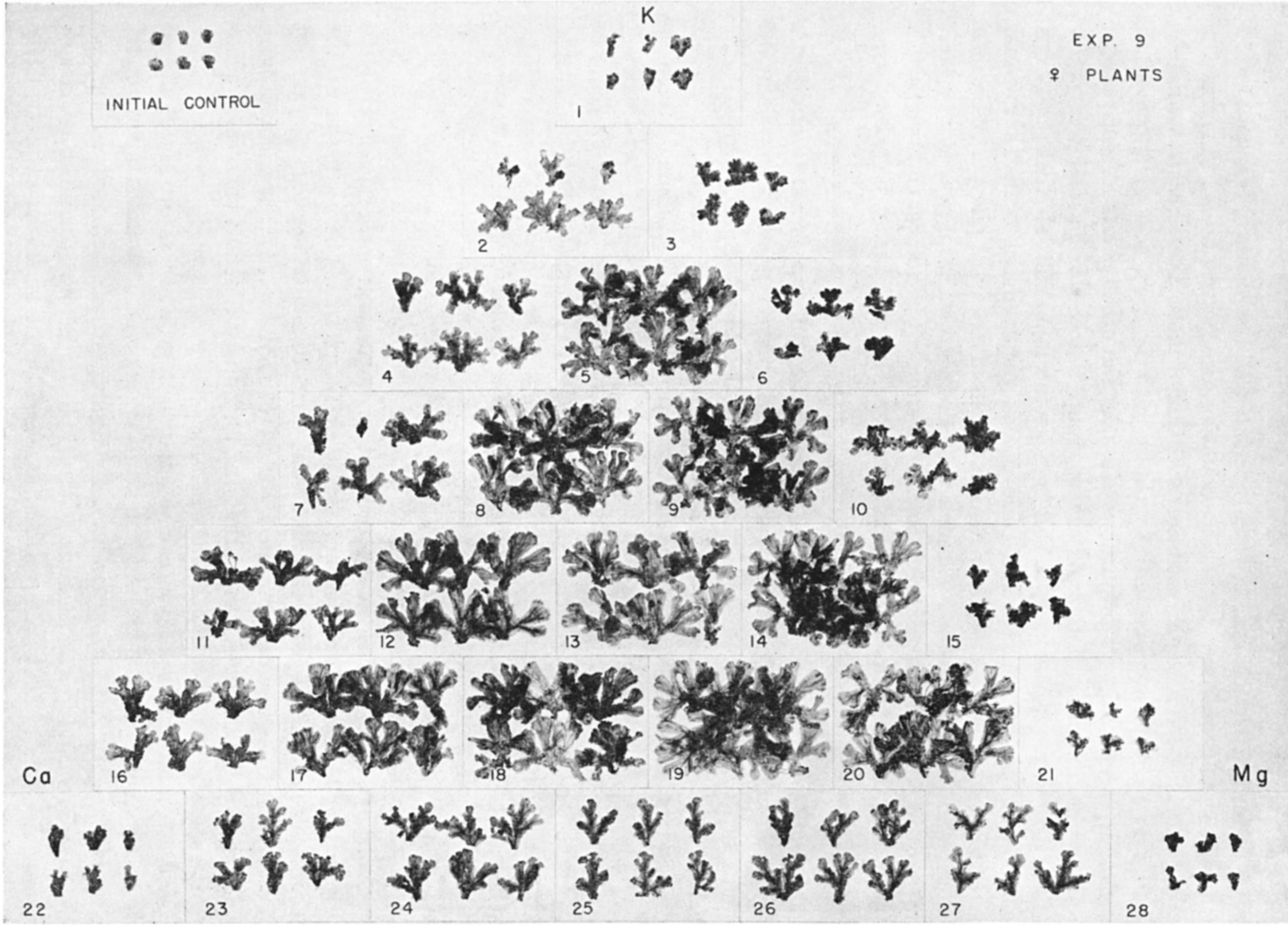

FIG. 5.—Cation triangle of experiment 9, May, 1940; archegonial plants on long photoperiod. Large amount of growth on some combinations necessitated overlapping the plants for photographing.

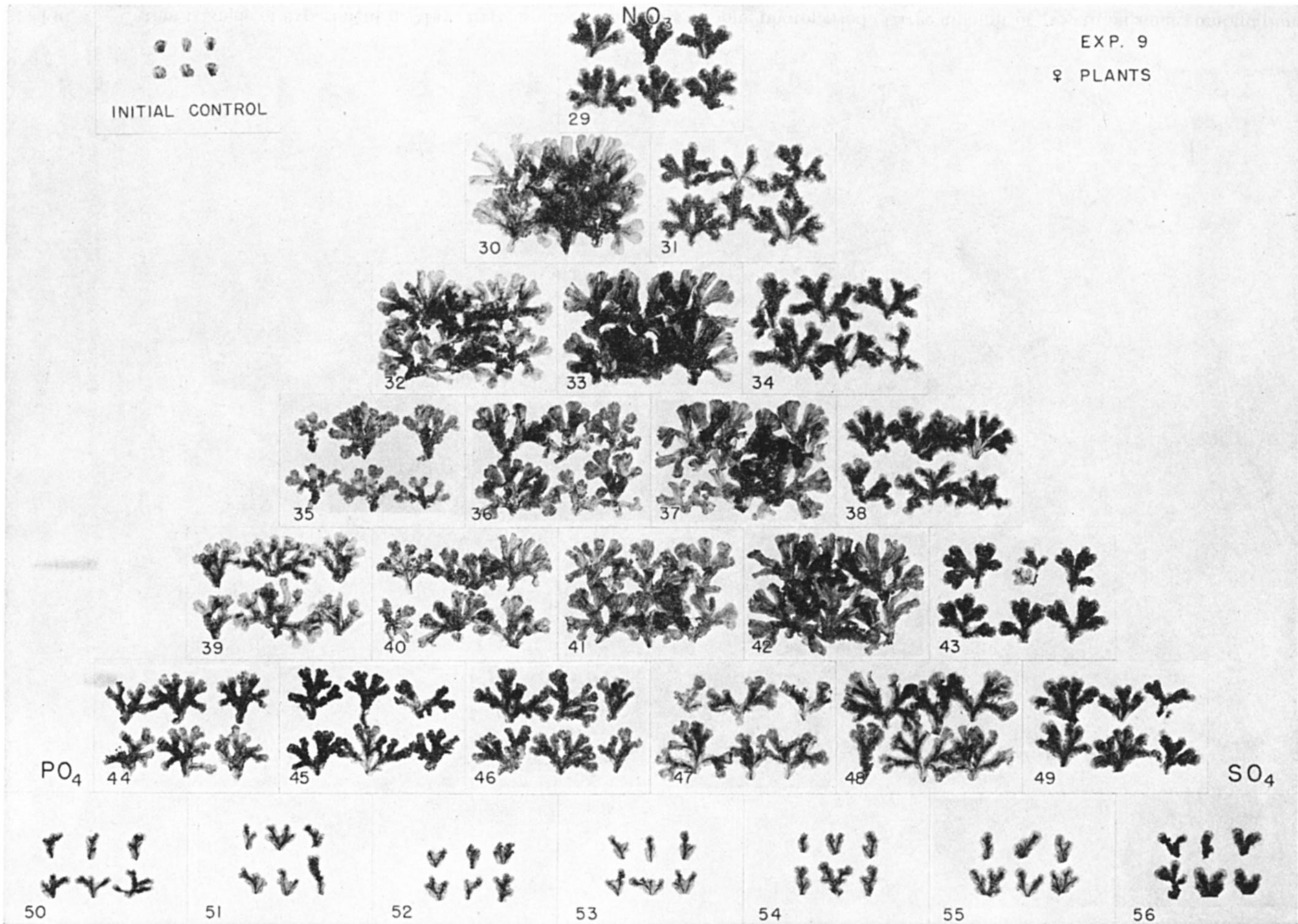

Fig. 6.—Anion triangle of experiment 9, May, 1940; archegonial plants on long photoperiod. Large amount of growth on some combinations necessitated overlapping the plants for photographing.

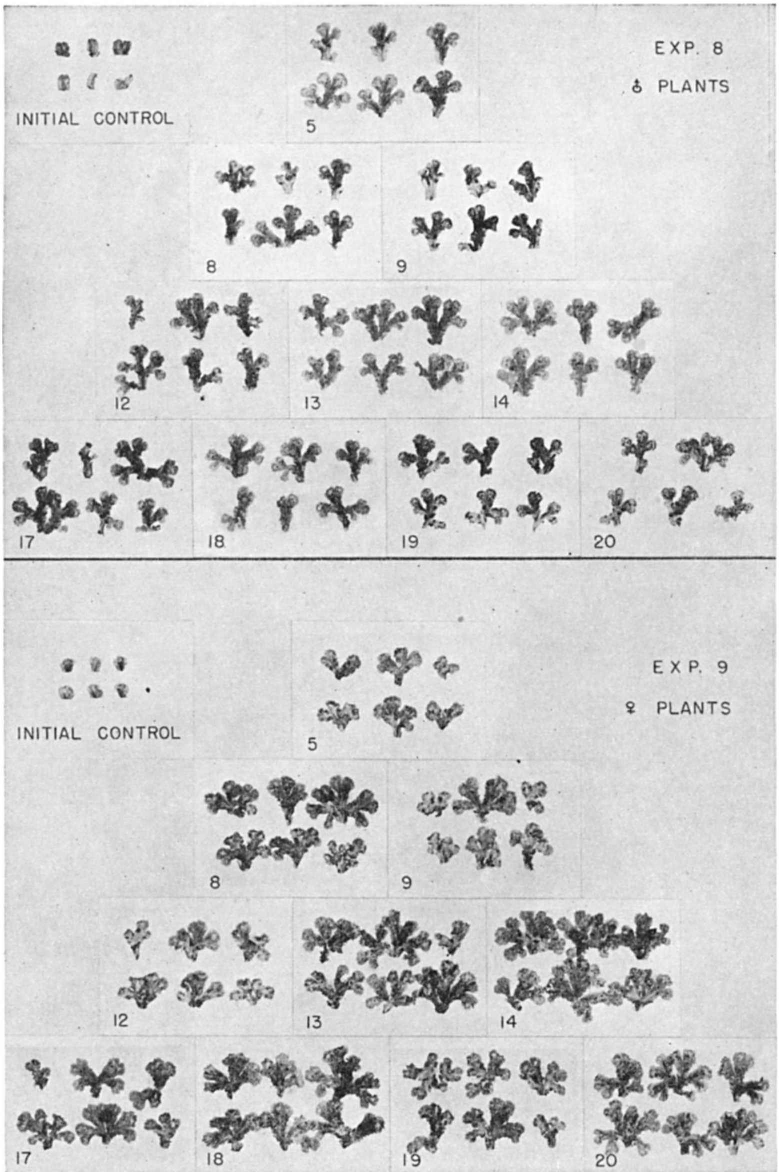

FIG. 7.—Cation triangles of experiments 8 and 9 on short photoperiod. Antheridial (above) and archegonial (below) plants grown April and May, 1940, respectively. Numbers correspond to same combinations as indicated in figs. 3 and 5. Total growth is much less than under long photoperiod (*cf.* figs. 3 and 5; tables 2 and 3).

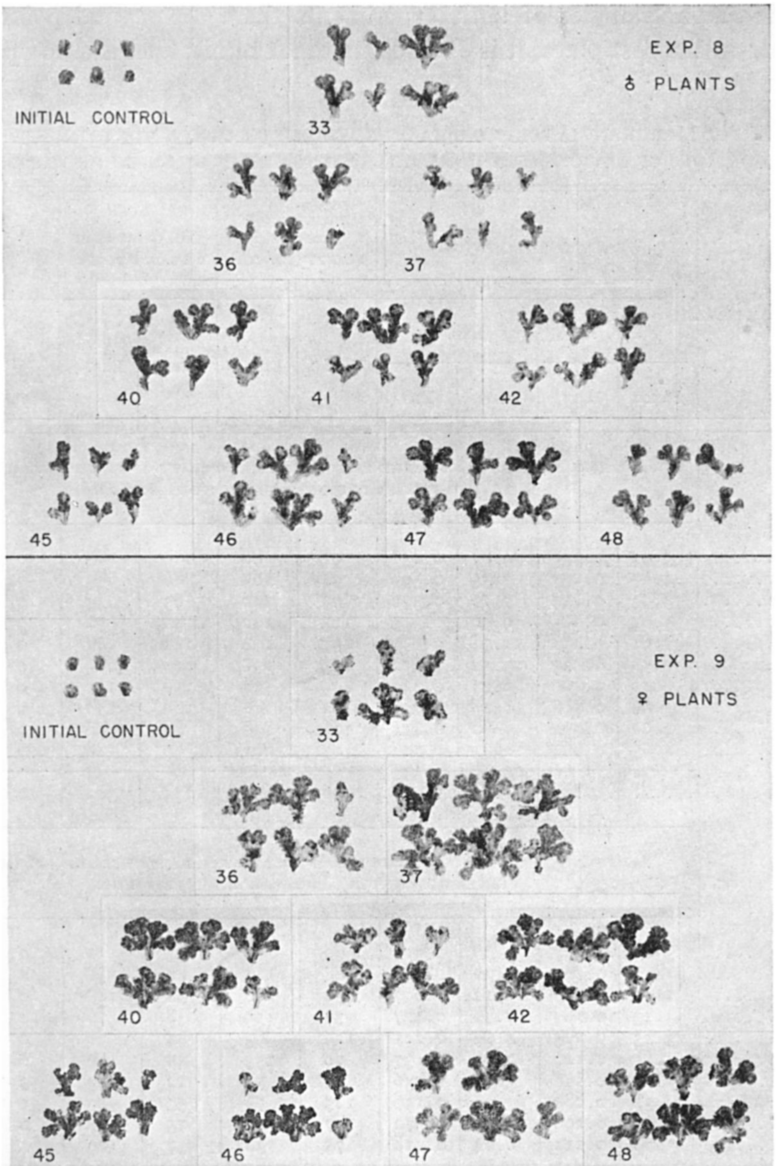

FIG. 8.—Anion triangles of experiments 8 and 9 on short photoperiod. Antheridial (above) and archegonial (below) plants grown April and May, 1940, respectively. Under short photoperiod the total growth is less than under long photoperiod.

In the anion triangles of both experiments there are several points of significance. In the first place, it is obvious that lack of nitrogen is accompanied by

TABLE 3

GROWTH OF MARCHANTIA POLYMORPHA ON NUTRIENT SOLUTIONS CONTAINING VARIOUS PROPORTIONS OF CATIONS OR ANIONS. ALL PLANTS GROWN ON SHORT PHOTOPERIOD

Position in triangle	Weight of 6 plants (gm.)				Area of 6 plants (sq. cm.)		No. of gemmae cups on 6 plants		No. of gametangiophores on 6 plants	
	Exp. 8		Exp. 9							
	Fresh	Dry	Fresh	Dry	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8 Antheridiophores	Exp. 9 Archegoniophores
	Cations K, Ca, and Mg varied proportionately on basis of sixths (cf. table 1) Anions present in constant relative proportion									
5	5.91	0.330	4.87	0.250	91	88	110	64	0	6
8	4.67	0.271	9.55	0.509	94	165	86	153	0	1
9	3.94	0.239	7.20	0.376	93	116	83	75	0	1
12	5.04	0.365	6.50	0.354	113	112	98	52	2	3
13	7.01	0.451	11.98	0.670	137	194	143	177	0	0
14	6.56	0.445	13.52	0.854	133	238	120	223	0	2
17	6.80	0.460	6.94	0.441	125	137	116	110	0	0
18	5.65	0.378	11.48	0.732	112	202	108	172	0	4
19	4.66	0.294	7.60	0.451	101	149	81	98	0	0
20	4.03*	0.274*	10.41	0.704	87*	201	90*	188	0*	2
Totals	....	....	....	....	1086	1602	1035	1312	2	19
	Anions NO_3, PO_4, and SO_4 varied proportionately on basis of sixths (cf. table 1) Cations present in constant relative proportion									
33	3.64	0.257	3.76	0.226	90	70	53	31	0	3
36	2.54	0.191	5.30	0.344	68	114	44	58	2	2
37	1.60	0.127	11.12	0.637	50	208	38	183	0	2
40	3.23	0.185	7.70	0.427	77	155	58	76	1	3
41	3.15	0.215	4.09	0.227	128	93	44	31	0	3
42	3.61	0.199	7.69	0.437	78	155	49	109	0	5
45	1.88	0.118	3.62	0.250	45	79	12	42	3	2
46	4.32	0.282	3.28	0.199	125	76	53	19	1	0
47	5.15	0.296	5.55*	0.372*	107	113*	75	76*	0	3*
48	3.08	0.211	6.94	0.418	75	138	57	109	4	3
Totals	....	....	....	....	843	1201	483	734	11	26

* Triangle position 20 in experiment 8 and triangle position 47 in experiment 9 possessed only 5 plants each at time of harvest. Had 6 plants been present it is assumed that all figures given would have been about one-fifth greater.

only slight growth, regardless of the content of phosphate or sulphate. It is also obvious that most growth occurred on those solutions low in phosphate and high in nitrate; that is, in solutions 30, 33, and 37. Considerable growth was also produced on solutions 42 and 48. Thus apparently a more marked effect was evident in relation to extremely small variations in the phosphate content than to any other single anion. The rate of accumulation of dry matter was apparently favored by low phosphorus content. Abundant phosphorus, particularly in those solutions where the nitrate content was low, was associated with decreased growth. Lack of sulphur resulted in decrease in the rate of growth on those solutions where the phosphate content was high but did not greatly affect the rate of growth on those solutions where the phosphate content was low (and the nitrate content high); for example, solutions 29 and 30.

More gemmae cups were produced on the antheridial than on the archegonial plants in both triangles (table 2). In the cation triangles more gemmae cups were produced on the solutions occupying a central position, particularly solutions 5 and 9, which were relatively low in calcium and magnesium and relatively high in potassium. Fewer gemmae cups were produced when there was an absence of potassium. In the anion triangles the production of gemmae cups was strikingly correlated with the phosphorus content of the solution. In both experiments more gemmae cups were produced on the solutions lacking in phosphorus than on any other, and the fewest gemmae cups were produced on the solutions lacking in nitrogen. There seemed little correlation between gemmae cup formation and sulphur content of the solution. Although in many combinations in both experiments numerous gemmae came to rest on the glass cloth and were permitted to remain relatively undisturbed for many days, none grew to form a mature thallus. Most gemmae disintegrated or were washed off the cloth when the solutions were changed.

The number of antheridiophores or archegoniophores produced on the various solutions was not clearly correlated with the concentration of any particular ion. In both experiments these structures were produced throughout both triangles. In the anion triangle of experiment 8 there seemed to be more antheridiophores on the solutions lacking phosphorus than on any other, although the correlation is not high. The determining factor in formation of antheridiophores and archegoniophores seems to be length of photoperiod rather than character of nutrient solution.

The total area produced by the plants growing on the various solutions was correlated rather closely with the accumulation of dry weight (tables 2, 3). Also, much greater growth was produced on certain solutions in the anion triangles of both experiments than was produced anywhere in the cation triangles. This is particularly true for solutions 30, 33, and 37, such solutions being relatively low in phosphorus. It may be that the phosphorus content of the cation triangles was slightly too high for maximum growth. If one wished to find a nutrient solution most favorable for the growth of this particular plant, consideration of the various results recorded here would indicate that such a solution should have a relatively low phosphorus content and a relatively high nitrogen content. But to produce the maximum development of antheridiophores or archegoniophores, or of gemmae cups, it might be necessary to utilize in the nutrient solution an amount of phosphorus approximating 0.00015 mol per liter or less.

Comparative effects of long and short photoperiod on growth of Marchantia

As already noted, at the time experiments 8 and 9 were started, comparable plants were placed on solutions 5, 8, 9, 12, 13, 14, 17, 18, 19, and 20 and solutions 33, 36, 37, 40, 41, 42, 45, 46, 47, and 48, and exposed to conditions of short photoperiod. The area, fresh and dry weight accumulation, gemmae cup formation, and gametangiophore production are recorded in table 3.

The growth and accumulation of dry matter by these plants was much less than under conditions of long photoperiod. Practically no antheridiophores or archegoniophores were produced by any of the plants exposed to short photoperiod, and those which were produced were usually found in the older portions of the thalli and were probably already initiated at the time the experiment was started. The area of these plants paralleled closely their relative rate of accumulation of dry weight and was much less than comparable plants under conditions of long photoperiod. All plants exposed to short photoperiod produced an abundance of gemmae cups, and while the total number of cups produced by any particular set was in certain cases about the same as those produced by comparable plants exposed to long photoperiod, the number produced per unit area was always far greater. On most solutions the total number of gemmae cups produced by any particular group of six plants—growing on a specific nutrient solution and exposed to short photoperiod—far exceeded comparable plants exposed to long photoperiod. This is true in spite of the fact that the total area of these plants and their total dry weight were much less. On short photoperiod archegonial plants produced a greater total number of gemmae cups than did antheridial plants (table 3).

Internal anatomy

In experiments 8 and 9, one of the six thalli of each treatment was transferred to killing fluid immediately after the fresh weight of the lot was determined. After fixation, a representative thallus fork was selected for anatomical study. A piece of plant body, extending about 4 mm. from the apical notch toward the base of the plant, was trimmed so that its width approximated 2.5 mm. Immediately adjacent, a cross section of the thallus approximately 5 mm. wide was removed. Both plant fragments were imbedded in paraffin and sectioned at 7 μ. All sections were vertical; those through the apex longitudinal, and those through the more nearly mature part of the plant were transverse with respect to the axis of the thallus. The longitudinal sections had in most instances meristematic, maturing and mature tissues. The cross sections had only relatively mature cells and tissues. Close microscopic examination of all preparations was made to determine whether any differences were apparent and whether such differences were possibly related to variations in nutrient supply and photoperiod.

Aside from such differences as width of thallus, coloration of scales and lower epidermis, development of rhizoids, and rapidity of dichotomy—characteristics which may be more readily determined by gross examination—very few striking anatomical differences were observable in the microscopic sections. Whenever the apical region of *Marchantia* was active mitotically, the number of cell divisions of the respective segments of the apical cells was about constant. Cell counts were made on both types of sections on nearly all the preserved plants. Although the thickness of the thallus varied considerably, the number of cells in a cross section was relatively constant. Counts were made vertically in a region twenty cells back of the apical cells in the longitudinal sections and in the midrib of the cross sections. Few thalli deviated from a cell count of 16 to 20; the greatest variation was from 11 to 26. Only the storage tissue was included in the count, to obviate the difficulties in recognizing cells in the photosynthetic areas in necrotic thalli. Plants growing on short photoperiod or on a solution deficient in one or two ions were thin vertically compared with plants growing on long photoperiod and a solution containing all the ions used in this experiment. Such thinner plants almost invariably possessed as many cells vertically as those thalli which appeared to be thick, the difference being in the size of the cells. Plants growing on solutions where necrosis occurred were thin in vertical distance and in the number of cells as counted vertically.

Anatomical effects of nutrient deficiencies

The results of anatomical studies are based on more than 1000 slides of serial sections. On long photoperiod some plants received solutions lacking in one or more cations or anions.

Potassium deficiency.—Plants grown in solutions very low in potassium possess no single anatomical alteration or condition attributable to such deficiency. Plants from solutions of the cation triangle numbering 22 to 28 inclusive (fig. 2) showed the customary anatomical development when a balance between calcium and magnesium was maintained. Necrosis was very marked in 22 (fig. 10*A*) and 28 (fig. 12*C*), the former lacking in calcium and the latter in magnesium (in addition to the absence of potassium), where large areas or nearly the entire thallus was nonliving (fig. 12*C*). Local necrosis was evident in 23 and 24 in female plants (grown in May). Plants on solutions 25, 26, and 27 were not necrotic except for the lower tip of the female plant in longitudinal section on solution 26. Anatomically the contrast between the plants on combination 27 and the nearly lifeless plants in position 28 is most striking. The former received one-sixth of the calcium solution and five-sixths of the magnesium solution, while the latter received the six-sixths magnesium solution only. In general, plants in the row lacking potassium stored more starch than plants in any other treatment, with the possible exception of the plants in the row lacking magnesium. Cell walls were not noticeably thick, but no plasmolysis occurred during fixation. Cells of the photosynthetic filaments were relatively large, distended, and possessed few chloroplasts. Pegged and smooth rhizoids were abundant.

CALCIUM DEFICIENCY.—The most striking anatomical changes were observed in relation to calcium deficiency. Apical growth in such plants ceases after a few days, and the usually green growing point becomes translucent and soon blackens. Large areas of such plants (1, 3, 6, 10, 15, 21, and 28) are necrotic, possess few rhizoids, and regenerate new thalli from the lower surface in the region of the midrib. Since regeneration was induced only in the plants lacking calcium, and since the development of new thalli occurs with such regularity, details will be discussed later in this paper. Death of the tips of the thallus was most rapid in combinations 21 and 28, which received relatively large percentages of magnesium. Regeneration occurred with greater regularity in 1, 3, and 6, becoming less frequent in 10, 15, and 21. Only one plant on combination 28 regenerated a thallus. Plants lacking in calcium generally possessed large upper epidermal and relatively few photosynthetic cells, which remained distended even when a layer of algae (mainly *Chlamydomonas*) completely covered the upper surface of the plant. These cells of the photosynthetic layer usually were devoid of chloroplasts.

MAGNESIUM DEFICIENCY.—Plants grown on combinations 1, 2, 4, 7, 11, 16, and 22 showed magnesium deficiency. The first and last combinations were also lacking in calcium and potassium, respectively. Plants in both treatments were necrotic and on combination 1 regenerated. Plants on the remaining solutions comprising the row in which magnesium was lacking were comparable with control plants in appearance, except that the mature upper epidermis and the mature photosynthetic layers were collapsed. Cells of these regions were well fixed when near the growing point, and immature. As seen in longitudinal sections there was a gradient of turgid cells to collapsed ones in the upper part of the thallus. Starch storage was pronounced in all treatments. The lower epidermis was thick walled and stained readily with safranin.

NITROGEN DEFICIENCY.—Characteristic growth responses were evident in the lowest row of the anion triangle. All plants were stunted, were light yellow in hue and deep red or purplish red underneath. This opaque red color was present in all the older scales and in the lower layers of the thallus, except the last 5 mm. of the tip, which was fairly translucent. The red color persisted after plants were preserved for nearly a year in F.A.A. solution (fig. 9*A*). The aquatic form or variety of *Marchantia* is described as possessing on the upper surface a median band pigmented with purple, associated with the absence of air chambers (7). Colored cell walls in liverworts have been ascribed to strong light combined with rather high temperature (11). MÖBIUS (25) reports that anthocyanin is present in the cell walls of *Marchantia* and persists for many years, even in a glycerin mount.

In longitudinal section (fig. 10*B*) the rather short zone of meristematic cells at the tip passes rather abruptly into the mature region of the thallus, which is characterized by large storage cells. Plants on a solution lacking in nitrogen were the first to develop rhizoids and were firmly attached to the glass cloth in a few days. No microscopic evidence was found of initiation of rhizoids nearer the tips. Dichotomy in plants lacking in nitrogen was infrequent, so that the narrowly elongated thalli were easily recognizable. Cross sections usually possess only one midrib (fig. 9*A*). Stored starch was evident in only a few of the plants, and all cell walls were thicker than in plants receiving all ions. The plant selected from combination 50 in experiment 9 was the only one showing a necrotic area among the anion plants. In a few scattered combinations, such as 10 and 16 in experiment 8 and 48 in experiment 9, under long photoperiod the lower epidermis and a few adjacent layers were relatively thick walled, but not as prominently so as in the nitrogen deficient plants.

PHOSPHORUS DEFICIENCY.—In these experiments plants grew well and to large size on solutions containing very small amounts of phosphorus. In fact there was considerable development, even though the solution contained no phosphorus—apparently a sufficient amount being present in the growing tips used for purposes of propagation at the beginning of the various experiments. The midribs of the thalli appeared black. Near harvest time it was obvious that the black coloration was a combination of the red lower surface and the dark green of the rest of the thallus. The older scales and the lower part of the thallus were red, much as in the nitrogen deficient plants, but possibly more intense. This color also persisted in F.A.A. solutions and in freehand sections was found to be limited to scales, rhizoids, lower epidermis, and at most the three cell layers nearest the lower epidermis. In the absence of fresh material it is presumed that the red color is the same for the plants when nitrogen or phosphorus is absent. The number of smooth rhizoids was very abundant (fig. 10*C*). In the midrib region pegged rhizoids often formed a bundle having a diameter which exceeded the width of the thallus. In the storage region of some plants lacking phosphorus (in experiment 9) cells were apparently plasmolyzed (fig. 10*C*).

SULPHUR DEFICIENCY.—Combinations 29, 30, 32, 35, 39, 44, and 50 contained no sulphur. Solutions 29 and 50 also lacked phosphorus and nitrogen, respectively, and the plants possessed the anatomical characteristics of these deficiencies.

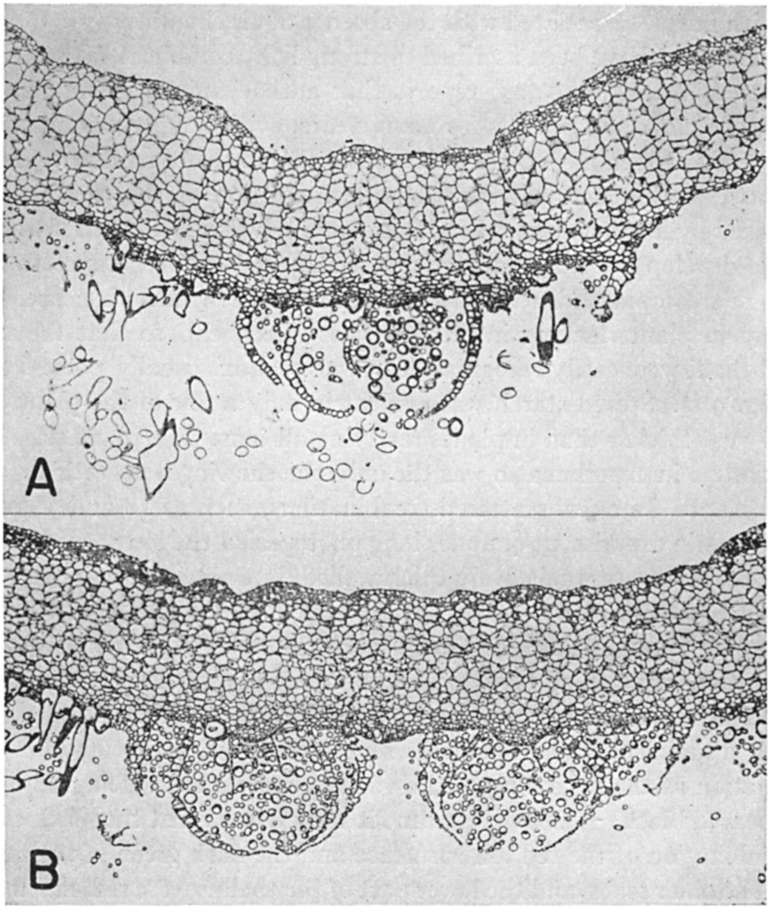

FIG. 9.—Cross sections of thalli in midrib region: *A*, nitrogen deficient plant on long photoperiod, experiment 8, on solution 55; cells of scales, rhizoids, lower epidermis, and adjacent layers are thick walled and colored red. *B*, initial control plant; darker staining cells below photosynthetic layer of thallus shown enlarged in fig. 14*A*.

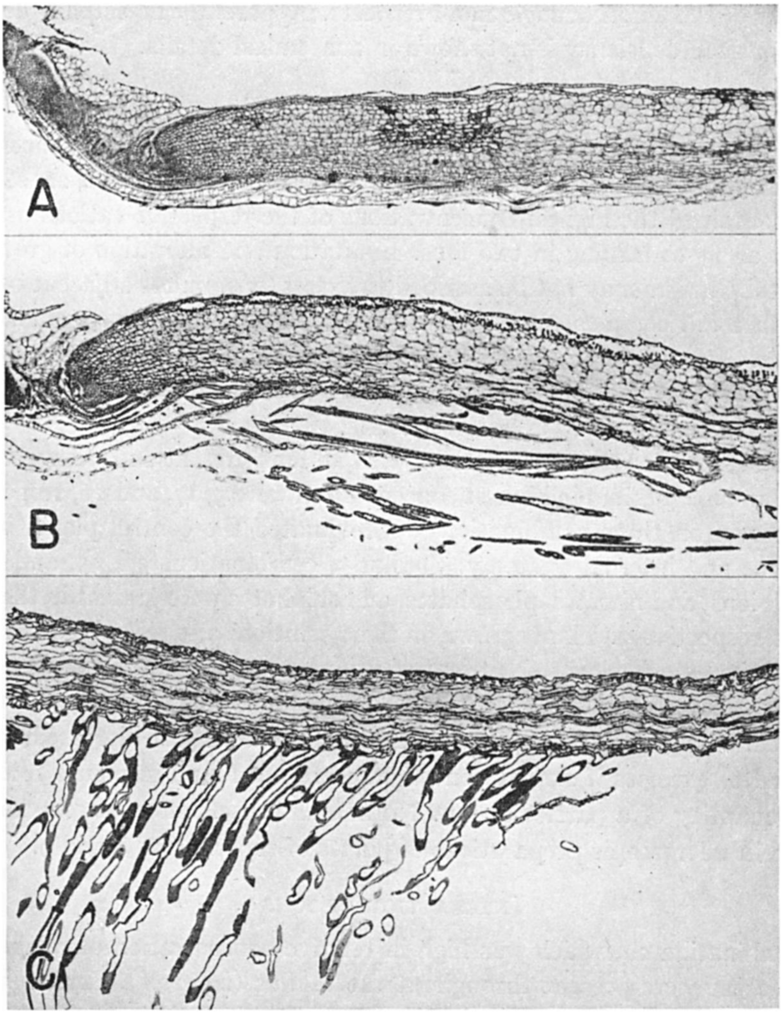

FIG. 10.—Vertical, longitudinal sections of thalli in midrib region: *A*, plant grown on combination 22 lacking potassium and magnesium but having relatively high concentration of calcium; experiment 8 on long photoperiod; several necrotic areas visible. *B*, plant grown on combination 54 lacking nitrates; experiment 9 on long photoperiod; mature region passes abruptly into less differentiated zone near tip. *C*, plant grown on combination 34 lacking phosphates; experiment 9 on long photoperiod; smooth rhizoids numerous.

Cross and longitudinal sections of plants on solutions 30, 32, 35, 39, and 44 resemble the sections of initial control plants and plants located in combinations in the center of the anion triangle in all respects. Apparently no sulphur deficiency existed, or such deficiency is not shown in anatomical details.

Effects of excess ions

Because of the nature of the experimental conditions, the greatest concentration of any ion was at the apices of the triangles (fig. 2). Combinations 1, 22, 28, 29, 50, and 56 contained the highest concentrations of the respective cations or anions, but each also was lacking in two ions. Limitation and alteration of growth and anatomical changes may not be ascribed to excess ions, unless adjacent combinations with a full complement of ions in some proportion also have similar characteristics. If comparable conditions are not present in the latter, it is reasonable to assign characteristics of growth and structure in the plants at the apices of the triangle to lack and not to excess of ions.

In the cation triangle, combinations with all ions present and with potassium, calcium, and magnesium in highest concentration were 5, 17, and 20, respectively. Plants grown on these combinations approximated the control plants in gross appearance and internal anatomy. Similarly, combinations 33, 45, and 48 contained all ions, and nitrates, phosphates, and sulphates were present in the largest amounts, respectively. Plants grown on these solutions also resembled the control or the original stock plants. Nutrient solutions of greater salt content, and consequently greater osmotic concentration, could be employed. Any appreciable anatomical change in plants grown on the combinations just discussed could be attributed to excesses if similar conditions were not found in plants receiving a smaller quantity of a particular ion. No anatomical effects of excess ions were observed in microscopic preparations of plants of experiments 8 and 9.

Interaction of ions

In combination 22, which was high in relative concentration of calcium ions, necrotic areas were present throughout the thallus (fig. 10*A*). Similar but less marked necrosis was also present in plants grown on solutions 23 and 24. These also were on relatively high calcium solutions, five-sixths and four-sixths, respectively. Plants on combination 26 were nearly devoid of necrosis, and thalli on 25 and 27 lacked necrotic areas entirely. Plants on solution 28 were almost wholly necrotic (fig. 12*C*). Since all combinations (22 to 28) lacked potassium, such diversity of anatomical expression cannot be ascribed to its absence alone. These combinations varied conversely in calcium and magnesium content, and it is likely that high calcium supply accompanied by low magnesium supply—together with lack of potassium—favored necrosis. High magnesium supply accompanied with varied calcium supply (one-sixth to three-sixths) and absence of potassium favored the usual anatomical development in *Marchantia*.

In the row of nutrient combinations lacking calcium (1, 3, 6, 10, 15, 21, and 28), low magnesium supply and attendant high potassium supply favored thallus regeneration, but a nutrient supply with converse proportions of these cations retarded the formation of adventitious thalli.

No interrelationships of anatomical effect on plants were noted with the cation magnesium and the other cations, and none of the anions interacted to produce an anatomical change great enough to be observed.

Anatomy of plants supplied with all nutrient ions in various proportions

Plants on combinations 5, 8, 9, 12, 13, 14, 17, 18, 19, and 20 in experiments 8 and 9 are very similar anatomically. Such plants grew large in area, elongated rapidly (at times even elongating their gemmae cups), and possessed rather brittle thalli. As a rule the upper epidermis was shrunken in microscopic preparations, and the photosynthetic filaments were partially collapsed. Walls of cells in the central portion of the storage region were so thin that many cells collapsed, forming a strandlike zone running longitudinally through the thallus. Scales are formed at the apex of the plant but are shrunken and inconspicuous in the mature region. Both types of rhizoids develop as in control plants.

In the anion triangle, combinations receiving all ions in various proportions were 33, 36, 37, 40, 41, 42, 45, 46, 47, and 48. Again the anatomy of plants in experiments 8 and 9 is very similar. As a rule the cells and tissues in these plants were comparable with plants supplied with all ions in the cation triangle. No single characteristic nor any obvious set of criteria was found on which to separate plants in these twenty positions from one another. Antheridial plants in experiment 8 and archegonial plants in experiment 9 reacted similarly.

Anatomical effects of short photoperiod

Anatomically the effect of short photoperiod on plants in both triangles, in both experiments, was identical. Only the twenty combinations just mentioned were used on short photoperiod. All plants appeared thinner in vertical section than plants in similar positions on long photoperiod. Cell counts in cross section in the storage region averaged 16 to 20, a range that duplicates the cell count in plants on long photoperiod. In the storage region of these plants the upper 5–7 cell layers averaged $40–50 \times 110–125\ \mu$ per cell. Cells in the next 4–6 layers averaged $20 \times 75–115\ \mu$. Cells nearest the lower epidermis and several layers above averaged $10–20 \times 40\ \mu$. The long axis of all cells was parallel to the axis of the thallus. The uppermost layer of storage cells averaged larger in size than comparable cells

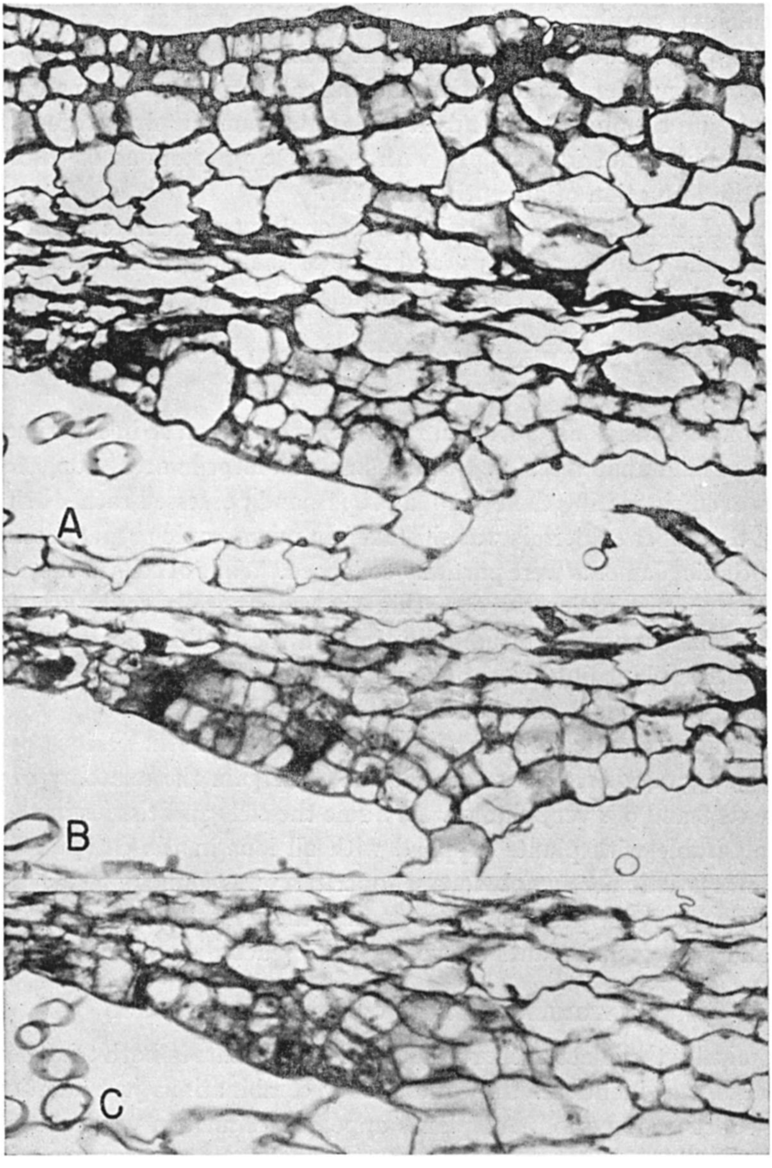

FIG. 11.—Vertical, longitudinal sections of thalli, experiment 8, combination 3, on long photoperiod: *A*, at left, bases of two rhizoids; *B*, similar section. Epidermal and subepidermal cells between bases of rhizoids have become meristematic; *C*, similar section showing more advanced meristematic condition, beginning of regeneration of new thallus.

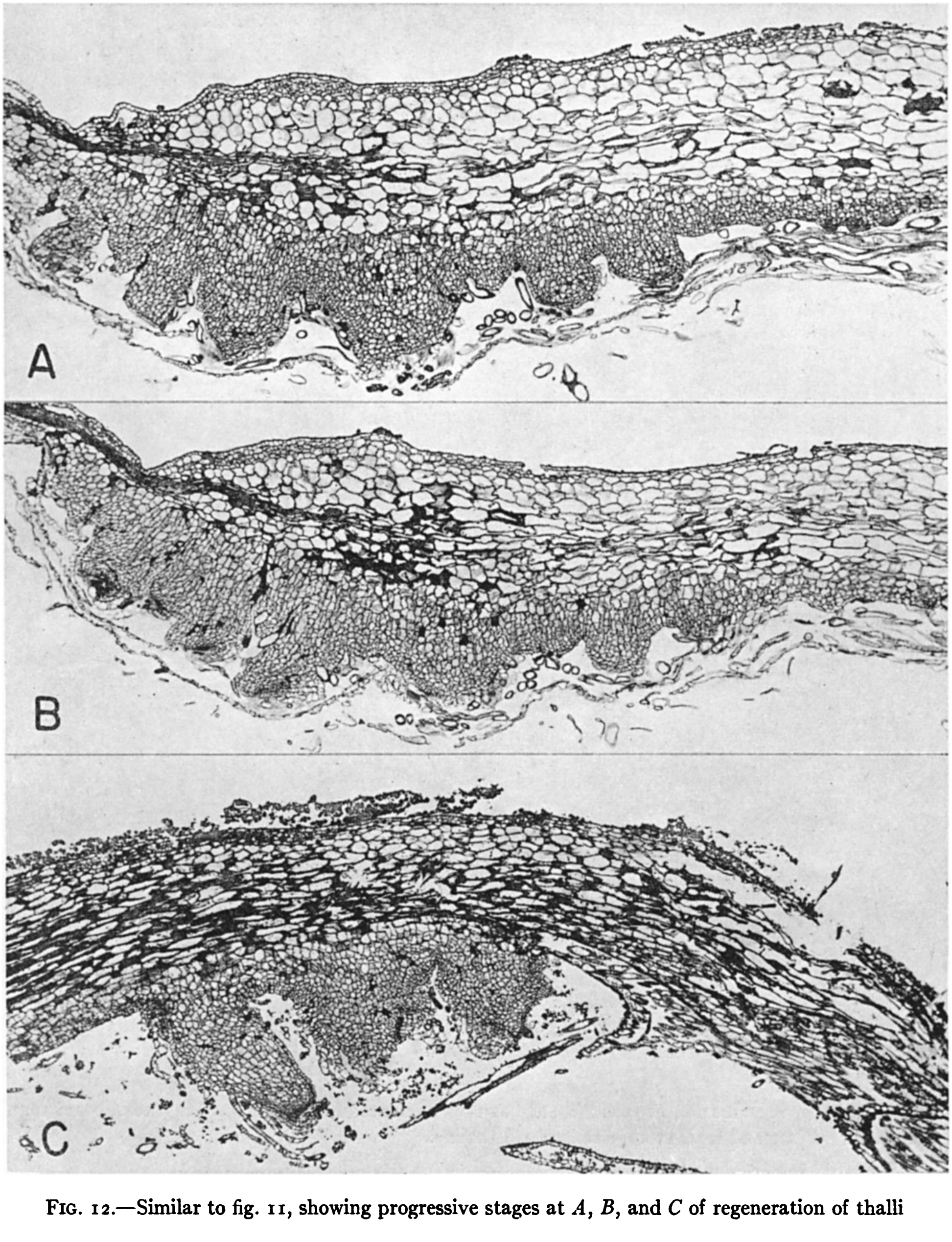

FIG. 12.—Similar to fig. 11, showing progressive stages at *A*, *B*, and *C* of regeneration of thalli

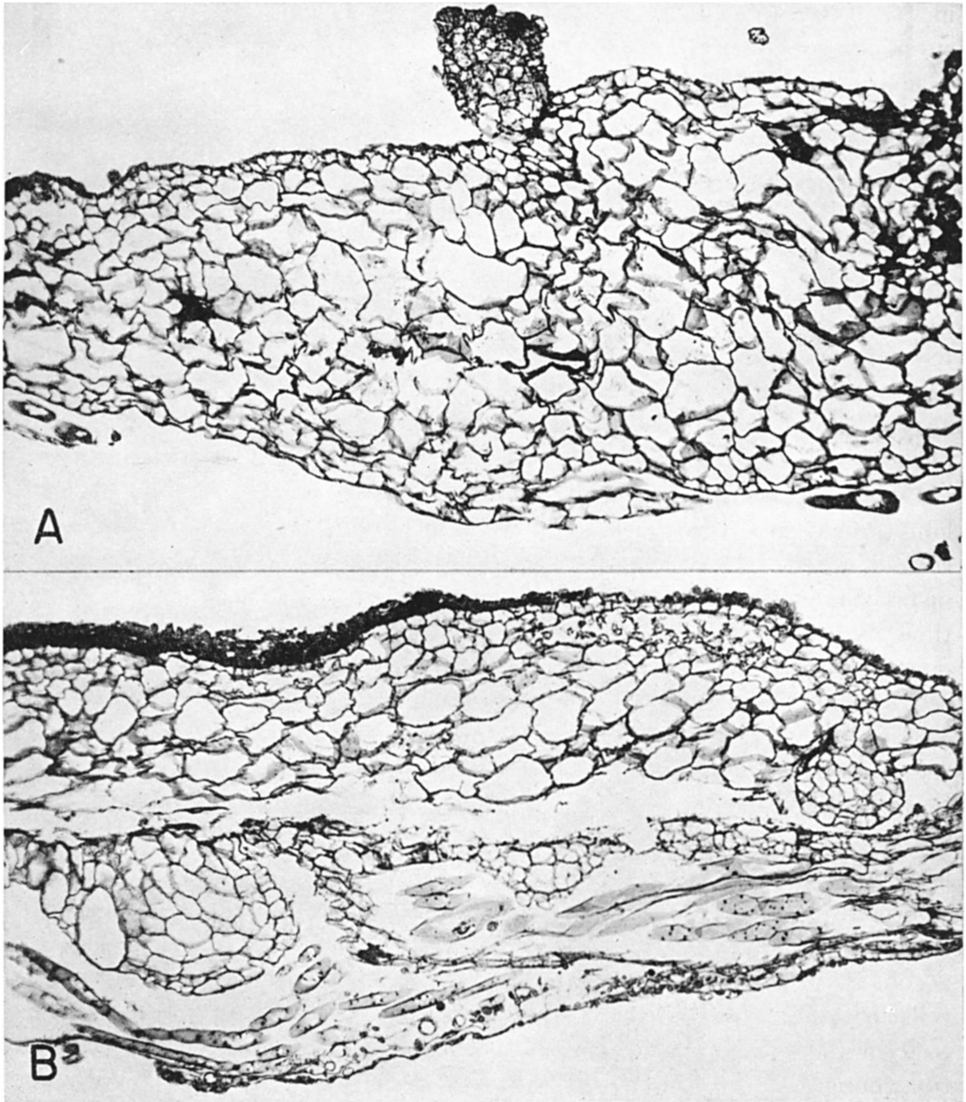

FIG. 13.—*A*, regeneration of young thallus at upper surface of old thallus; *B*, regeneration of thalli from lower surface and from storage cells of old thallus.

in any of the other treatments. The smaller dimensions of the other layers in the storage region, however, reduced the total thickness of the thallus. Gemmae cups in many stages of development were found in nearly all plants sectioned.

Lack of calcium and regeneration of thallus

All combinations in both experiments in which calcium was lacking (1, 3, 6, 10, 15, 21, and 28) regenerated new thalli from the underside of the midrib region. Although such regenerated thalli never became very large, they were visible to the unaided eye. On solutions 1, 3, and 6, numerous thalli were formed; in some cases a regenerated thallus in turn regenerated another one. Fewer thalli were formed on combinations 10, 15, 21, and 28; on the latter, only one of the sectioned plants in experiment 9 possessed a regenerated thallus. Seven-μ sections of *Marchantia* plants on these combinations presented a series of developmental stages of new thalli (figs. 11–13).

Cells of the lower epidermis on either side of the midrib region, in interrupted longitudinal rows, may differentiate into large, dark-staining cells characteristic of rhizoid initials. These elongate as smooth rhizoids when the apical region has outdistanced them by about twenty cells, although some initials remain dormant for a time. When mature (fig. 9*A*, *B*), such rhizoids are smooth walled, grow vertically downward, and attach to the substrate (**7, 13**). The base of a rhizoid is large, and cells surrounding it usually undergo nuclear and cell divisions but do not enlarge appreciably. The rhizoid base has the appearance of being sunken two or three cell layers into the lower side of the thallus (fig. 11*A*). In plants grown on solutions lacking calcium, these small epidermal and subepidermal cells surrounding the base of the rhizoid become meristematic (fig. 11*B*). A small group of cells is formed which protrudes from the lower level of the epidermis (fig. 11*C*). Initiation of such meristematic groups occurs in basipetal succession (fig. 12*A*). The meristematic group of cells soon differentiates a mucilage hair (slime papilla), accompanied by a small, acropetally placed depression. In this depression, apical cells are differentiated. Groups of such growing points often occur simultaneously to form a longitudinal row of thalli (fig. 12*A*, *B*, *C*). In median longitudinal section young thalli have the same structure as adult plants, except that meristematic activity is limited to the apical cells and one subepidermal row (fig. 12*B*, *C*). The tips of all regenerated thalli are directed toward the tip of the parent plant and may emerge from beneath it. In all plants regenerating ventrally, only the lower epidermis—and at most about five cell layers of the lower part of the thallus—are involved in the production of new thalli.

Two types of exceptions to the method of regeneration just described are shown in figure 13. The uppermost thallus has produced an adventitious bud from the upper epidermis (combination 10, experiment 8). Only one other such example

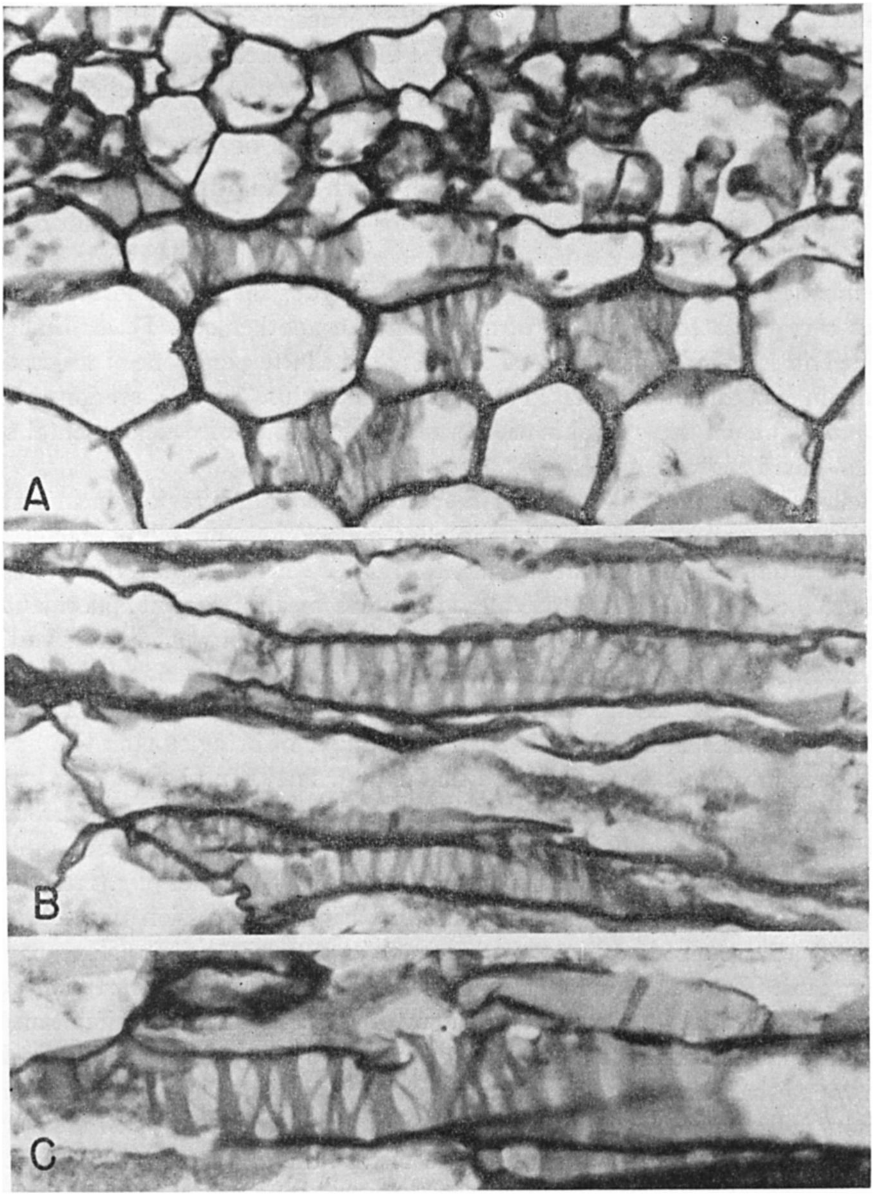

Fig. 14.—Cells of storage region showing wall thickenings as commonly found in all cultures: *A*, transverse section; *B*, *C*, longitudinal sections.

was found (combination 3, experiment 9). In this variation of regeneration only a few upper layers of cells were involved in the production of the new thallus. Because of the absence of air chambers (fig. 13*A*), it is known that the bud was formed in the midrib region which in this species commonly lacks air chambers.

The second type of exception to the usual method of regeneration was found in one thallus only (combination 3, experiment 9). Necrosis had occurred in a longitudinal layer of the storage tissue, a few cells above the lower epidermis (fig. 13*B*). A part of this necrotic strand disintegrated, leaving an air space. Storage cells forming the roof of this space became active and formed a small, internal bud. The parent thallus also produced a bud from the lower epidermis in the usual manner (fig. 13*B*).

KLEIN (20) reports that *Marchantia polymorpha* growing naturally in Hungary regenerated from the furrows of the archegoniophores. Several investigators have shown that in thallus cuttings of thallose liverworts regeneration proceeds from the ventral surface of the apical end (2, 6, 21, 36). VÖCHTING (36) illustrates the younger stages of regeneration in the furrow of the archegoniophore of *Marchantia* following injury. The absence of adventitious bud formation on all nutrient combinations containing only the smallest fraction of the calcium solution indicates that the absence of calcium or the interaction of factors in the absence of calcium may act to stimulate certain ventral cells to become active.

STORAGE CELLS WITH WALL THICKENINGS AND PITS

On every nutrient combination and under both photoperiods, *Marchantia* plants possessed elongated cells in the storage region which were thickened on the inside face of their walls. At times these thickenings were very faint and resembled protoplasmic strands. End walls of storage cells of the initial controls possessed scalariform thickenings (fig. 14*A*). Such thickenings varied in width (fig. 14*B*, *C*). These cells were found either near the photosynthetic tissues (fig. 14*A*), in the middle of the thallus, or very near the lower epidermis (especially on solution 56). They were most prominent in longitudinal sections near the midrib and appear to have been living cells at the time of fixation, since starch grains were present in several such cells (combinations 16 and 11 in experiments 8 and 9, respectively). In necrotic thalli such as those on combinations 10, 15, 21, and 28, these thick-walled cells stained deeply with safranin, which is characteristic of nearly all the nonliving cells. Cells from plants lacking in calcium possessed thickenings which covered most of the wall, leaving only large oval pits.

Storage cells with walls thickened in various ways have been repeatedly mentioned by bryologists for *Marchantia* (3, 7, 12, 18, 26, 35, 37), *Conocephalum* (*Fegatella*) (2, 4, 27), *Lunularia* (32), *Monoclea* (30), *Bryum* and *Mnium* (15), and members of the Dilaenaceae such as *Symphyogyna* (22). Isolated drawings of such cells are presented by BOLLETER (2), CAVERS (4), and SCHIFFNER (32) for *Conocephalum* and *Lunularia*, respectively. GOLENKIN (14) figures mycorrhizal hyphae inhabiting red-violet storage cells in *Preissia*, *Marchantia palmata* and *M. paleacea*, all of which are said to be pitted in age. The tracheids found by HOLLOWAY (19) in the gametophyte of *Psilotum* probably belong to a category different from those described in the accompanying figures.

General discussion

While the foregoing results are based upon relatively few plants on each combination of nutrients, some of the data are striking and clear cut. Growth of *Marchantia* is greater on nutrient solutions with a low osmotic concentration than on those of a relatively high one. The effect of length of photoperiod is also marked; vegetative extension, gametangiophore production, and vegetative activity are greater under long photoperiod than short photoperiod, although under the latter condition formation of gemmae cups is increased. This seems true regardless of the nutrient solution upon which the plants are grown. In our experience it has also been found that the particular strains of *M. polymorpha* used will grow very rapidly, even under conditions of relatively high light intensity and relatively high greenhouse temperatures, dependent upon the composition of the nutrient solution supplied. It has been found repeatedly in different experiments that plants growing on the various nutrient solutions produced much greater growth than did comparable plants growing on garden loam soil, even though the plants on such soil were abundantly supplied with water. On some of the nutrient combinations the plants grew and gained more dry weight than did plants observed under field conditions at comparable seasons of the year. The only plants which have been observed growing as rapidly and as large in natural habitats were found on areas from which vegetation had recently been burned or on the charcoal and ash residues of burned trash piles.

The marked effects resulting from variations in the proportions of the major cations and anions indicate clearly that *Marchantia* produces much greater growth on nutrient solutions relatively low in phosphorus content, regardless of great variation in sulphate and nitrate content. It is likely that with certain combinations even greater growth may be produced. If, for example, larger triangles were used, so that the variations were not so great from one combination to another, and if the number of plants grown on each particular solution were greater, a more rigorous statistical analysis of the results could be made. It is highly desirable to establish cation triangles in which much lower concentrations of phosphorus are present, and it would be equally desirable to test higher concentrations of nitrate. Under such range of combinations in the cation triangle, it might well be that small differences in the content of potassium, calcium, or magnesium would show even more striking effects than were obtained in the present experiments. The method used is applicable to the general study of problems of mineral nutrition.

Summary

1. *Marchantia* grows readily on glass cloth in an open moist chamber when supplied with an appropriate nutrient solution. A method for growing it under controlled conditions of nutrient supply and photoperiod is described. A male and a female strain of *Marchantia polymorpha* were employed, each strain propagated vegetatively from a stock grown from a single plant.

2. A range of fifty-six nutrient solutions, each having an osmotic concentration of approximately 0.285 atmospheres, was used. The cations involved were K, Ca, and Mg, and the anions NO_3, PO_4, and SO_4.

3. Cultures containing the cations K and Mg but without Ca all regenerate new thalli, formed in the main from adventitious buds arising from cells on the ventral side of the thallus at the midrib region. In cultures lacking NO_3 and PO_4 the ventral layers of cells develop a red-violet color in the walls. The absence of Mg and SO_4 does not result in so striking a response as lack of the other ions. Phosphate is required in very small quantities. Up to the limits of the quantities supplied, plants make greater growth with increasing amounts of NO_3, provided all other ions are present.

4. Some of the plants were grown on long photoperiod, 18 hours of light out of each 24-hour cycle, and on short photoperiod, 9 hours of light out of each 24. The differences between the lots of plants grown on the two photoperiods were both quantitative and qualitative. Irrespective of the range of nutrient supply, plants on long photoperiod were larger and had greater dry weight than similar plants on comparable solutions on short photoperiod. Irrespective of the range of nutrient supply, plants on short photoperiod produced a greater total number of gemmae cups but on long photoperiod a greater number of gametangiophores than comparable plants on short photoperiod.

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