Effects of Nutrient-Solution Concentration on the Growth of Marchantia polymorpha
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EFFECTS OF NUTRIENT-SOLUTION CONCENTRATION ON THE GROWTH OF MARCHANTIA POLYMORPHA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 552

PAUL D. VOTH

Introduction

Following forest fires, and even burns of local extent, *Marchantia polymorpha* commonly invades the bare area, forming an almost pure stand (**6**, and others). Dry heat is known to make available greater quantities of solutes, which may inhibit green plants such as oats but stimulate the growth of *Pyronema*, a fungus (**23**). Since little is known concerning the concentration of a complete nutrient solution which would be optimum for a liverwort such as *Marchantia*, the following experiments were performed.

In tests conducted as preliminary to those already reported (**31**, **32**), it was established that a complete nutrient solution with an osmotic concentration of about 0.75–1.00 atmospheres, as used in greenhouse culture of common vegetable plants, was not conducive to maximum growth of *Marchantia*. At that time (1939) it was estimated that a dilution of approximately one-fifth was a more desirable solution. The present report is apparently the first to determine the dilution which is optimum for the growth of this species of *Marchantia*. Since the relative concentrations of the common ions had already been determined in this laboratory, a series of solutions were devised in which the relative proportions of the various salts remained constant but the amount varied according to a definite plan.

The problem of varying the concentration of a balanced or complete nutrient solution, with consequent changes in osmotic concentration, diffusion rates, and total salts available has been considered for many years. Experiments concerned with varying the amount of one or two of the constituents of a nutrient solution usually introduce several additional problems (**15**). Results when the amount of salts is changed proportionately must be judged by the range of concentration employed, the kind of plant, the season of the trials, the substrate used (whether sand or liquid culture), and other factors. A summary of such investigations has been made by TOTTINGHAM (**29**). LIVINGSTON (**15**), SHIVE (**24**), and others have reported earlier experiments dealing with the effect of varying the concentration of all salts. In general, it was found that the weaker or intermediate nutrient solutions produced plants superior in size or dry weight (**1**, 713.4 p.p.m.; **2**, 0.3 per cent "normal" solution; **3**, 300 p.p.m. in water culture, 2500 p.p.m. in sand; **9**, 1.5 atmospheres of pressure; **10**, 0.6 atmospheres or less; **20**, 1/50 dilution of Knop's solution; **22**, 2880 p.p.m.; **25**, 1.75 atmospheres; and **27**, salt concentration approximately equal to solution 5 of present report). Plants utilized for these experiments were tobacco, oats, wheat, tomato, barley, duckweeds, buckwheat, and pineapple, with the cereal grains predominating. On the other hand, several workers maintain that the solutions with higher salt content produced optimum growth (**4**, 3000 p.p.m.; **7**, **8**, 1142 p.p.m.; **19**, 2200 p.p.m.; and **28**, all utilizing water cultures). It is noted that solutions considered concentrated by some are classed as intermediate by others. A few workers favor the view that the concentrations of nutritive salts can

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vary widely before any noticeable effect is demonstrated (**11**, 1433–5337 p.p.m.) or that such fluctuations are relatively unimportant (**13**). In several of the investigations just mentioned solutions leached from the soil were employed (**7, 8, 22**).

While dry weights of plants or plant parts and similar data are important in the determination of the optimum solution, anatomical investigations often supplement or even change the interpretation of data. LIVINGSTON (**15**) noted that the usual branched filament of the alga *Stigeoclonium* was supplanted by spherical colonies of cells when strong solutions of inorganic salts were used as a growing medium. SCHOENE (**21**) demonstrated that no rhizoids are produced on newly-formed protonemata of *Funaria* and other mosses. The most critical studies of NIGHTINGALE and FARNHAM (**16**) established that dilute nutrient solutions resulted in vigorously growing, succulent sweet-pea plants. The failure of cells to mature rapidly, especially at the base of the pedicel, was responsible for the formation of an abscission layer and subsequent loss of the flower. HAYWARD and LONG (**9**) demonstrated that in tomato high nutrient concentrations favored rapid maturation, especially of vascular tissues, with consequent smaller cells and organs.

A correlation between gross appearance, weights, area, and anatomical changes of *Marchantia* is the object of the experiments reported here.

Material and methods

Clones of male and female plants of *Marchantia polymorpha*, designated cultures A and B, respectively (**32**), were again used in these experiments. The culture method described previously (**31**) consisted of placing six plants on a disk of glass cloth supported by a glass rack and placed in the open half of a large moist chamber. Plants and apparatus were pressure-rinsed with distilled water daily, after which they were supplied with 150 cc. of the appropriate nutrient solution. The present investigation is designated experiment 11 and was conducted in the greenhouse from March 29 to April 25–26, 1941 (27 days). A photoperiod of 17 hours was provided by supplementing daylight with 200-watt Mazda filament bulbs in reflectors (**32**). The bulbs were automatically lighted from 6:00 P.M. to midnight.

The basic solution was determined by means of nutrient triangle experiments and was suggested in a previous paper (**32**, p. 325). This solution is designated number 5 in table 1. By using higher as well as lower relative proportions of the fundamental solution (number 5), ten solutions were prepared with the extremes of concentrations ranging from 160 to 1. Solution 2 is of approximately the same concentration as nutrient solutions used to grow greenhouse plants such as beans, tomatoes, etc. Solution 1 is twice as concentrated as solution 2; and beginning with solution 6, succeeding dilutions are by one-half.

Osmotic concentrations were determined cryoscopically, using freshly prepared solutions. The hydrogen-ion concentrations were made with an instrument equipped with a glass electrode. The higher dilutions tended to be slightly more acid than less dilute ones, probably because of the acidity of the distilled water. When left standing for several weeks in covered, glazed earthenware containers, the solutions tended to become more alkaline, with proportionally larger increases as dilutions were greater.

The molar concentration of solution 5 is: K 0.0012, Ca 0.0007, Mg 0.0014, NO_3

0.0034, PO_4 0.0004, and SO_4 0.0008 mols per liter. These ten solutions did not remain stable when plants were actively growing on them. Owing to the high nitrate content and the low phosphate concentration, the hydrogen-ion concentration decreased rapidly soon after the solution was supplied to the plant. In several stock cultures, readings of pH 10.0 or greater were recorded without pronounced detrimental effects to the plants. This tendency toward alkalinity is well known (**30**). When a solution can be used in which KH_2PO_4 or other phosphate salts (**17**) are relatively high, and even greater in amount than any other salt (**26**), this fluctuation in pH is greatly diminished by the buffer action of the phosphate.

TABLE 1

QUALITIES OF NUTRIENT SOLUTION EMPLOYED IN EXPERIMENT II (MARCH–APRIL, 1941)

Solution no.	Relative concentration of solutions		Molar concentration of salts (millimols)					Concentration of micronutrients (p.p.m.)	Osmotic concentration in atmospheres (average)	pH (average)
	Decimal	Fractional	KNO_3	$Ca(NO_3)_2 \cdot 4H_2O$	$Mg(NO_3)_2 \cdot 6H_2O$	KH_2PO_4	$MgSO_4 \cdot 7H_2O$			
1....	1.00	160/160	8.0	7.0	6.0	4.0	8.0	2.0*	1.83	4.93
2....	0.50	80/160	4.0	3.5	3.0	2.0	4.0	1.0	0.94	5.14
3....	0.30	48/160	2.4	2.1	1.8	1.2	2.4	0.6	0.59	5.38
4....	0.20	32/160	1.6	1.4	1.2	0.8	1.6	0.4	0.37	5.66
5....	0.10	16/160	0.8	0.7	0.6	0.4	0.8	0.2	0.21	5.94
6....	0.075	12/160	0.6	0.525	0.45	0.3	0.6	0.15	0.18	5.92
7....	0.050	8/160	0.4	0.350	0.30	0.2	0.4	0.10	0.13	6.05
8....	0.025	4/160	0.2	0.175	0.15	0.1	0.2	0.05	0.08	6.03
9....	0.0125	2/160	0.1	0.0875	0.075	0.05	0.1	0.025	0.05	6.09
10....	0.00625	1/160	0.05	0.04375	0.0375	0.025	0.05	0.0125	0.01	5.95

* Concentration of $MnSO_4$, $ZnCl_2$, and $Na_2B_4O_7$, singly; concentration of $FeSO_4$ is 0.1 of the figure given.

Investigation

Weight, area, and gemmae cups

Each *Marchantia* cutting, consisting of one apical notch and surrounding tissue, averaged 3 sq. cm. in area when planted on March 29. Each group of six plants received daily portions of 150 cc. of solution 5 for the first 3 days, since it was known that this nutrient combination promoted rhizoid formation. It has been used successfully for several years in growing the stock plants. Following this preliminary treatment, the specified variations of this solution (table 1) were supplied each culture of six plants again at a rate of 150 cc. per day.

After about the tenth day, plants supplied with solutions 1 and 2 became slightly translucent and were definitely smaller than plants on more dilute solutions. Plants on solutions 9 and 10 gradually developed red-purple scales, rhizoids, and ventral epidermis after a similar period. The development of translucency in plants grown on nutrient solutions of high concentration and the anthocyanin coloration of plants supplied with solutions of lesser salt content continued until the end of the experiment. Plants growing on solutions containing intermediate amounts of salts were generally the light to dark green color characteristic of *Marchantia*. The color and the general aspect of the plants at the end of the experiment may be summarized as follows:

SOLUTION 1.—Thalli were watery; wings and even tips of many plants were dead. Tips of surviving plants grew into elongated thalli, resembling regenerated tips of overwintered or injured plants commonly seen in discarded stock cultures.

SOLUTION 2.—Low-lying plants were watery, others were bright yellow-green. Tips generally were broad, and all plants were alive.

SOLUTIONS 3, 4, 5.—With decreasing salt content the plants tended to increase in size, to become darker in color, to possess ascending tips, and to develop rhizoids more abundantly.

SOLUTIONS 6, 7, 8, 9.—The plants became increasingly darker red-purple (rhizoids, scales, and lower epidermis) and decreased slightly in size with decreased concentration of the solution. Rhizoids were produced in increasing quantities, and thalli were brash.

SOLUTION 10.—The excessive development of anthocyanin on the ventral surface and the yellow-green of the dorsal surface indicated that the lack of an adequate nitrate supply was more pronounced than the lack of phosphate. The smaller number of gemmae cups and the narrow thallus with few dichotomies also expressed nitrate more than phosphate deficiency. These plants much resembled those of previous experiments in which nitrates and phosphates were lacking (31).

Table 2 shows the various trends of growth. Fresh and dry weights tend to increase progressively from solutions 1 to 6 and to decrease from solutions 7 to 10. Plants grown on solution 5 had a total fresh weight of 18×8.518 gm., which was slightly higher than the weight of plants on solution 6 (18×8.096 gm.). The greater weight of plants on solution 5 in this instance may be attributed to the very favorable growth of all female plants on the latter solution. The fresh weights of the male plants and the calculated dry weights of both sexes were all greater when plants were growing on solution 6. When actual dry weights of plants growing on the two solutions are known and compared, both sexes produced more substance when grown on solution 5. Male plants in one culture dish supplied with solution 5 grew poorly, which, at least in part, accounts for the failure of solution 5 to be optimum by all methods of calculation.

In area, the female plants on solution 5 exceeded similar plants on solution 6. In all other comparisons of area, solution 6 is optimum to solution 5. As shown in table 2, male plants on solution 4 were slightly larger on the average than similar ones on solution 5 but did not approach the average area of male plants on solution 6.

Gemmae cups vary greatly from plant to plant, but a comparison of the totals of averages based on eighteen plants (table 2) indicates that the maximum number was produced on solution 4. For both

TABLE 2

GROWTH OF MARCHANTIA POLYMORPHA ON TEN NUTRIENT SOLUTIONS IN WHICH AMOUNTS OF CONSTITUENT SALTS VARIED PROPORTIONATELY (TABLE 1). FIGURES ARE AVERAGE FOR ONE PLANT, BASED ON EIGHTEEN PLANTS GROWN IN THREE CULTURE DISHES. MAXIMUM VARIATION IS GIVEN

Solution no. and sex	Weight (gm.)			Area (sq. cm.) (Original area 3 sq. cm.)	No. of gemmae cups	Ratio: weight (mg.) / area (sq. cm.)	
	Fresh	Dry				Dry‡	Fresh
		Calculated	Weighed				
1 ♂...	0.849 (±0.177)	0.0543 (±0.0032)	..........*........	16.11 (±6.11)	7.11 (± 5.89)†	3.89	53.20
1 ♂...	0.729 (±0.099)	0.0585 (±0.0073)	0.0611 (±0.0049)	13.55 (±5.45)	6.67 (± 3.33)		
1 ♀...	0.699 (±0.093)	0.0452 (±0.0113)	....	14.17 (±2.17)	2.44 (± 9.56)	3.84	54.60
1 ♀.	0.724 (±0.059)	0.0564 (±0.0144)	0.0550 (±0.0145)	11.89 (±4.28)	2.50 (± 6.50)		
2 ♂...	1.359 (±0.186)	0.0835 (±0.0187)	....	22.89 (±4.56)	9.83 (±11.17)	4.09	63.49
2 ♂...	1.502 (±0.539)	0.1017 (±0.0326)	0.1009 (±0.0328)	22.17 (±9.84)	13.72 (± 7.72)		
2 ♀...	1.507 (±0.272)	0.0861 (±0.0139)	....	25.33 (±2.84)	7.94 (±10.06)	3.72	64.92
2 ♀...	1.461 (±0.603)	0.0852 (±0.0272)	0.0841 (±0.0255)	20.39 (±8.56)	9.78 (±13.22)		
3 ♂...	1.768 (±0.155)	0.0954 (±0.0064)	....	29.78 (±0.95)	21.50 (± 9.50)	3.59	63.03
3 ♂...	2.059 (±0.431)	0.1231 (±0.0124)	0.1226 (±0.0137)	30.94 (±6.39)	22.33 (±13.33)		
3 ♀...	1.827 (±0.552)	0.0939 (±0.0247)	....	30.06 (±6.56)	16.61 (±18.39)	3.37	64.04
3 ♀...	1.845 (±0.750)	0.0999 (±0.0416)	0.0995 (±0.0402)	27.28 (±8.95)	14.44 (±14.44)		
4 ♂...	1.708 (±0.460)	0.0927 (±0.0214)	....	32.83 (±5.83)	20.22 (±14.78)	3.32	55.44
4 ♂...	2.114 (±0.445)	0.1355 (±0.0333)	0.1365 (±0.0318)	36.11 (±0.61)	24.22 (±13.78)		
4 ♀...	2.147 (±0.216)	0.1188 (±0.0137)	....	37.44 (±2.11)	21.33 (±16.67)	3.24	61.65
4 ♀...	2.473 (±0.120)	0.1237 (±0.0179)	0.1241 (±0.0154)	37.50 (±2.00)	17.72 (±15.72)		
5 ♂...	1.678 (±0.525)	0.1046 (±0.0296)	....	35.94 (±5.77)	20.22 (±13.22)	3.30	54.53
5 ♂...	1.996 (±0.484)	0.1164 (±0.0204)	0.1177 (±0.0196)	31.44 (±4.56)	18.56 (± 7.56)		
5 ♀...	2.176 (±0.473)	0.1244 (±0.0052)	....	39.44 (±2.44)	15.27 (± 9.27)	3.32	60.98
5 ♀...	2.668 (±0.662)	0.1458 (±0.0028)	0.1391 (±0.0131)	40.00 (±3.00)	11.11 (±11.89)		
6 ♂...	2.042 (±0.211)	0.1185 (±0.0107)	....	39.33 (±2.83)	20.67 (±12.33)	3.28	55.10
6 ♂...	1.809 (±0.186)	0.1156 (±0.0261)	0.1109 (±0.0333)	30.56 (±4.44)	15.39 (±12.39)		
6 ♀...	2.157 (±0.270)	0.1452 (±0.0300)	....	40.94 (±2.23)	9.94 (± 7.94)	3.54	54.35
6 ♀...	2.088 (±0.282)	0.1302 (±0.0110)	0.1314 (±0.0124)	37.17 (±3.17)	7.67 (± 7.67)		
7 ♂...	1.955 (±0.077)	0.1088 (±0.0072)	....	36.06 (±1.44)	18.11 (±12.11)	3.48	58.15
7 ♂...	1.999 (±0.178)	0.1277 (±0.0167)	0.1280 (±0.0165)	31.94 (±1.39)	16.78 (± 7.78)		
7 ♀...	2.272 (±0.074)	0.1232 (±0.0111)	....	40.11 (±1.06)	6.89 (± 8.11)	3.30	57.36
7 ♀...	2.052 (±0.521)	0.1262 (±0.0200)	0.1253 (±0.0183)	35.28 (±6.11)	4.11 (± 6.89)		
8 ♂...	1.809 (±0.108)	0.0981 (±0.0096)	....	34.00 (±3.17)	16.00 (±12.00)	3.18	53.76
8 ♂...	1.533 (±0.132)	0.1027 (±0.0090)	0.0996 (±0.0076)	28.17 (±1.67)	13.22 (±13.78)		
8 ♀...	2.027 (±0.352)	0.0964 (±0.0046)	....	34.06 (±1.27)	5.72 (± 8.28)	3.05	56.82
8 ♀...	1.976 (±0.193)	0.1184 (±0.0098)	0.1182 (±0.0101)	36.39 (±3.89)	4.06 (±10.94)		
9 ♂...	1.659 (±0.122)	0.0783 (±0.0052)	....	30.94 (±2.06)	10.11 (± 7.89)	2.97	49.98
9 ♂...	1.334 (±0.076)	0.0988 (±0.0087)	0.0994 (±0.0084)	28.94 (±3.06)	11.94 (± 7.94)		
9 ♀...	1.837 (±0.435)	0.0916 (±0.0031)	....	34.78 (±0.89)	5.17 (± 8.83)	3.15	51.94
9 ♀...	1.464 (±0.279)	0.1106 (±0.0071)	0.1089 (±0.0048)	28.78 (±1.22)	3.89 (± 7.11)		
10 ♂...	1.367 (±0.328)	0.0800 (±0.0117)	....	30.61 (±0.56)	5.89 (± 8.11)	3.03	51.21
10 ♂...	1.543 (±0.490)	0.0913 (±0.0093)	0.0923 (±0.0095)	26.22 (±3.05)	8.61 (± 7.39)		
10 ♀...	1.547 (±0.089)	0.0832 (±0.0173)	....	29.89 (±0.56)	1.33 (± 4.67)	3.07	52.75
10 ♀...	1.422 (±0.129)	0.0893 (±0.0112)	0.0896 (±0.0111)	26.39 (±3.39)	2.06 (± 4.94)		

* One plant from each of three culture dishes of each nutrient solution was preserved for anatomical study, hence no direct weighing of all dry plants in such lots could be made.

† Maximum variations are based on actual counts of gemmae cups on each plant.

‡ When available, the actual dry weight is utilized; otherwise the calculated dry weight is used.

sexes the numbers are greater for plants growing on solution 3 than on solution 5, but both treatments produced fewer gemmae cups than plants on solution 4. In all instances where totals from eighteen or more plants are compared for number of gemmae cups, the male plants of each treatment produced a greater number of these reproductive bodies than the female ones. In this experiment a total of 3060 gemmae cups was counted on female and 5420 on male plants. This ratio of 1 to 1.44 is less than the ratios of 1 to 4 and 1 to 6.6 reported for experiments 8 and 9 and experiment 10 (**31, 32**), respectively, where solutions approximated number 5 of this experiment with respect to osmotic concentration.

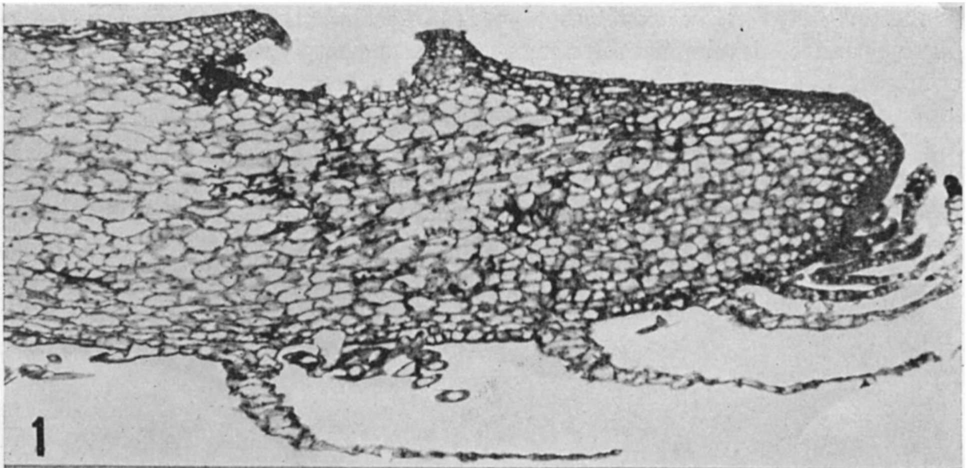
The ratios of the area to the weight, based on the averages of the plants of each sex, varied only moderately, with a tendency toward a higher ratio in plants grown on more concentrated solutions. The larger ratios (4.09 on the dry-, 64.92 on the fresh-weight basis) were found in plants of treatment 2, indicating a relatively smaller area and a relatively larger weight compared with plants growing on other treatments. Smallest ratios were secured for plants growing on treatments 8, 9, and 10.

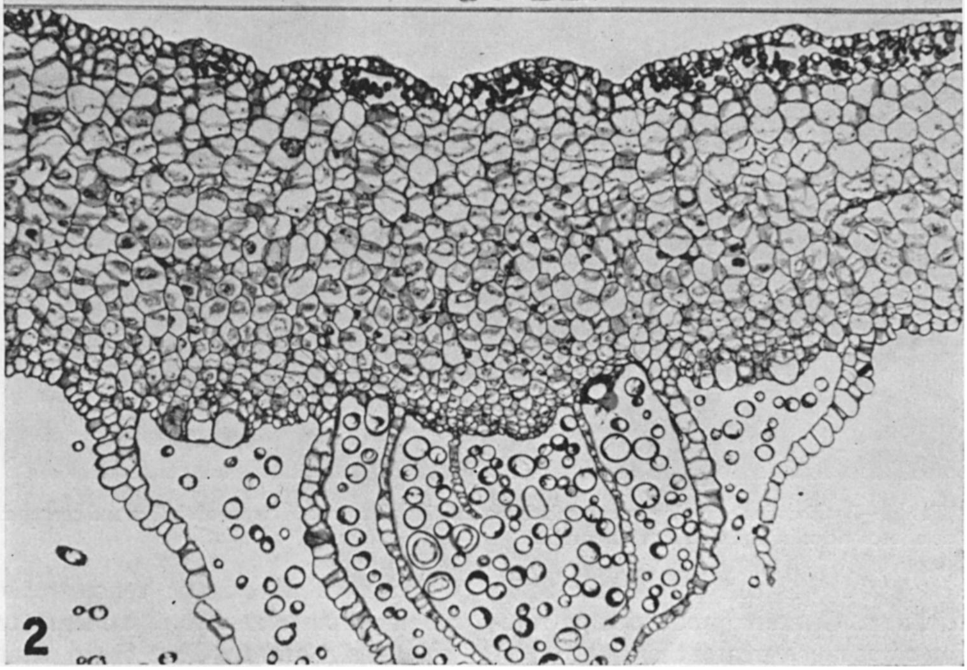
The ability of *Marchantia* to survive great changes in hydrogen-ion concentration is evidently shared by many mosses (**12**). Suitable buffers containing no phosphate (because of increased growth on solutions low in phosphates) should be added to nutrient solutions, thus controlling a factor which has not received enough attention in the experiments reported here.

Anatomical variations

Cross sections of the thallus, approximately 2 mm. back from the apical notch, and longitudinal sections through the notch, were mounted and stained by the usual procedure. Sections were 7μ in thickness. Sixty plants were investigated in this manner. Plants on concentrated nutrient solutions possessed thin cell walls which collapsed to a certain degree when preserved in formalin-acetic acid-alcohol. Figure 1 is a longitudinal section of a male plant on solution 1, passing through a gemma cup located dorsally. The apical cell is at the extreme right, just above the youngest scales. Ventrally, rhizoids and scales are distributed normally, although cells of the latter are collapsed. A very small zone of growth surrounding the apical cell contrasts with the remainder of the thallus, which has matured rapidly—as indicated by the optically empty cells. Many internal necrotic areas characterize this section and nearly all other plants growing on solution 1. The lack of gemmae in the cup and photosynthetic filaments in the air chambers is striking.

The other extreme of thallus development is shown in figure 2, a cross section of a female plant in the midrib region which was supplied with solution 10. It is characterized by optimum development of rhizoids of the smooth and tuberculate varieties, scales, storage region, photosynthetic filaments, and pores in the upper epidermis. Most cells have contents, and cell walls are unusually sturdy. No collapse of any cell is discernible. Dassonville (**5**) reported that seed plants grown in distilled water possessed a more prominent cuticle and fragile cell walls. Tissues matured and lignified rapidly and early, and secondary growth was limited. In contrast, *Marchantia* gametophytes develop thicker walls when only a very small amount of salt is present in the solution. Rapid and early maturity of tissues when plants are supplied concentrated solutions is equal-





FIGS. 1, 2.—Sections through thallus of *Marchantia* near growing tip: Fig. 1, longisection of male plant on solution 1 showing necrotic areas and lack of living gemmae. Fig. 2, cross section of female plant on solution 10 showing thickened cell walls and well-developed photosynthetic filaments.

ly true in *Marchantia*, sweet pea (16), and tomato (9).

Solutions between the extremes of 1 and 10 permitted development in a manner intermediate between that of the two plants illustrated. No necrotic areas were found in plants on solutions other than 1 and 2. As solutions became progressively more dilute, the collapse of cells lessened as cell walls increased in thickness. Gemmae production in solution 2 was much greater than in the most concentrated solution, and the number of cups reached a maximum in plants on solution 3. A survey of all microscopic sections in this experiment, as well as those of previous ones, indicates that—regardless of great variations in individual nutrient salts and variations in total salt content—*Marchantia* thalli produce a storage tissue 14–20 cells in thickness.

The extreme of cell derangement is shown in figure 3, where the optically empty cells of the older part of the thallus contrast sharply with the zones of necrotic cells nearer the apex.

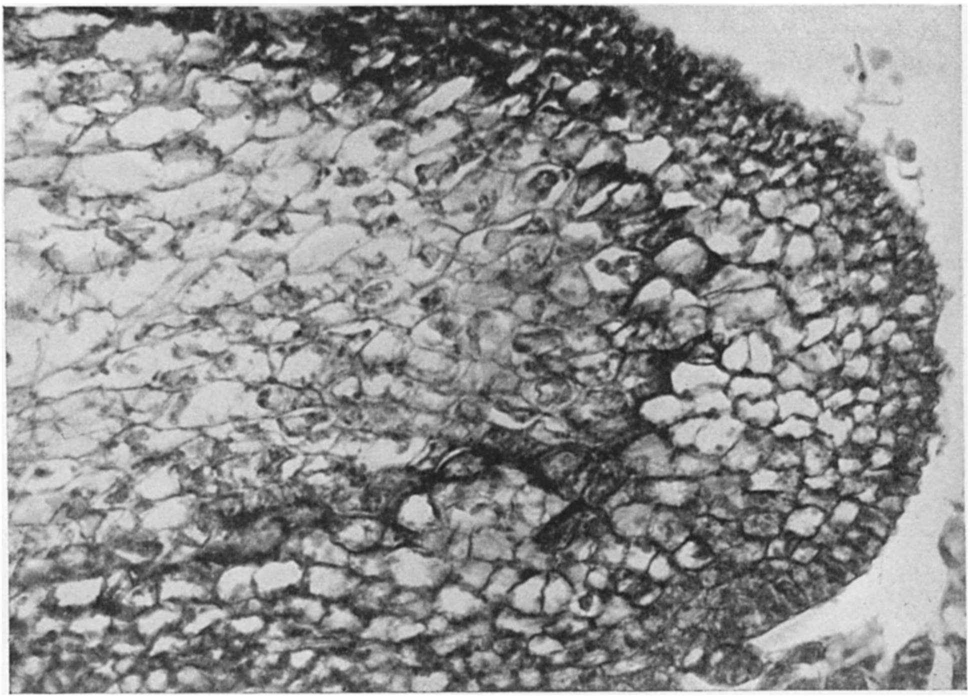


FIG. 3.—Longisection of male plant on solution 2 showing clear cells in center of thallus and concentric zone of necrotic cells. Apical cell at lower right.

Discussion

Marchantia polymorpha makes the greatest vegetative growth on solutions which have an osmotic concentration ranging from 0.21 to 0.18 atmospheres (solutions 5 and 6). That other plants grow larger on dilute solutions has been reported by SAEGER (20), who found optimum growth of duckweeds on a 1/10 dilution of Knop's solution, and by LIEBIG (14), who grew *Isoetes* from spores on diluted Knop's solution. That protonema of *Funaria* and *Ceratodon* can withstand solutions of high osmotic value is reported by PRINGSHEIM and PRINGS-

HEIM (18) and is evident from the report of SCHOENE (21). The exact conditions which *Marchantia* encounters when invading a newly-burned area has not been determined. From greenhouse experiments it is concluded that while this liverwort is hardy in extreme concentrations of nutrient solutions, its optimum vegetative development is in the dilute medium. The enormous size of some *Marchantia* plants in mountainous regions may depend on the attainment of such a dilution.

Solution 3, with an osmotic concentration of about 0.59 atmospheres, was most conducive to the development of gemmae cups (table 2). The larger number of cups on plants supplied with concentrated solutions was noted several years ago in preliminary experiments. Development of the cups evidently is dependent upon several factors when results of this and previous experiments are summarized. Four conditions contribute to greater production: (1) Relatively high osmotic concentration of the nutrient solution (table 2) results in the formation of a greater number of these reproductive bodies, especially when the smaller size of such plants is considered. (2) When phosphates are eliminated from the nutrient solutions and nitrates are abundantly supplied, a large number of cups are produced by relatively small plants (31, 32). (3) Male plants of clone A used for these experiments exceed female plants of clone B in production of the cups (table 2, and previous experiments, 31, 32). (4) More gemmae cups are produced on a short photoperiod of about 8 hours than on a long photoperiod of 17–18 hours (31).

If a common denominator exists for these factors it has not yet been determined for this plant.

Preserving one from each culture of six plants in previous experiments and from half the cultures in this one necessitated the selection of a representative plant for anatomical study and the calculation of the dry weight of six plants from the percentage dry weight of five plants and the known fresh weight of all. This method had not been checked for validity in the present series of experiments until experiment 11 permitted the culture of enough plants to make direct comparison possible. At harvest time half the cultures were treated as if one plant from each was to be preserved in an alcoholic solution. Instead it was segregated and its weight compared directly with the calculated weight (table 2). The greatest discrepancies between actual and calculated weights appeared in the averages of plants 5♀ and 6♂, with differences of 6.7 and 4.7 mg. per plant, respectively. In nearly all other cultures the dry-weight differences were only a milligram or less. About half the actual weights were higher and the other half lower than the calculated ones. With greater care in weighing fresh plants these differences should become even less.

Summary

1. Using methods previously described, cultures of two clones of *Marchantia polymorpha* were supplied with ten nutrient solutions ranging in concentration from 1.83 to 0.01 atmospheres, of which solution 5, with a concentration of 0.21 atmospheres and 0.0033 mols per liter, was optimum for vegetative growth —as judged by fresh and dry weights and area. It contained 1.6 cc. KNO_3, 1.4 cc. $Ca(NO_3)_2$, 1.2 cc. $Mg(NO_3)_2$, 0.8 cc. KH_2PO_4, and 1.6 cc. $MgSO_4$, each of a 0.5 M solution per liter, and the usual micro-nutrients. Solution 6 was by some standards slightly superior and by others inferior to solution 5. Its concen-

tration was 75 per cent that of solution 5. The pH characteristics of these solutions are briefly discussed.

2. Gemmae cups are most numerous on plants grown on solution 4.

3. Gemmae cups are produced on female and male plants in a ratio of 1 to 1.44—a lower ratio than in previous experiments.

4. Solutions with high salt concentration tended to kill the growing tips of plants and produced translucent thalli. Maturity was rapid, with cells slightly smaller than those grown on dilute solutions. Solutions low in salts favored the development of anthocyanin, numerous rhizoids, and a sturdy plant body possessing cells with living contents and thicker cell walls.

5. The calculation of dry weights of six plants from the weight of five and the fresh weights of six and five plants is reasonably accurate.

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