Some preliminary Experiments on Physiology of Charophyta.[1]

By V. Vouk and F. Benzinger.

The Charophyta have been frequently the object of study of the appearance of streaming of protoplasm, yet the fundamental questions regarding their physiology remained almost wholly untouched. The Charophyta, biologically, represent a fairly developed type among the Thallophyta. Regarding nutrition they remind us of higher plants because of the presence of rhizoids. According to Bierberg's investigations the rhizoids not only perform the function of fixations, but also the main function of nutrition. The nutritive materials are — to our knowledge — not yet analysed. Occasional observations only have been made so far, namely: that the growth of Charophyta varies in different soils (Migula, Ernst). We know just as little about the external factors of the growth of the Charophyta such as light and temperature. Only lately Karling investigated the activity of light. From observation in nature we know the great influence of light upon the growth and fructification of Charophyta.

The few investigations on Charophyta led to some preliminary fundamental experiments with Charophyta. The following questions were put:

1. How will Charophyta absorbe nutritive materials? Are the rhizoids the only organs of absorbtion or is the surface of thallus performing the same function?
2. What is the growth of Charophyta in differing soils?
3. What is the relation of the Charophyta to the light factor? In what way does light influence the growth of the vegetative organs and their propagation?
4. What is the relation of the Charophyta to mineral nutrition? Which mineral nutritive material is indispensable to Charophyta?

[1] This communication contains in condensed form part of the results of experimental work on Charophyta proposed by me and carried out by Mr. F. Benzinger in my laboratory under my supervision. At later time Mr. Benzinger will publish a series of further investigations of his own. Prof. Dr. V. Vouk.

These are but a few fundamental questions on physiology of Charophyta we have endeavoured to answer.

I. THE ABSORBTION OF NUTRITIVE MATERIALS.

Bierberg fully discussed the question in his experimental study on the significance of the plants-streaming for the transport of nutritive materials. He first reached the result that vital internodial cells of Chara and Nitella will admit no dyes nor any other lower molecular material trough its surface on any proven quantity. Only the membranes of very young internodial cells will admit certain diluted dyes.

It transpires from Bierberg's investigations that rhizoids of Chara actually not only perform the function of fixation of the plant in the substrate, but also essentially by supplying nutritive materials out of the soil. In this respect the rhizoids are to be compared with the most of the submerse Phanerogams rooted in soil, as established by Snell's careful experiments. On the other hand he proves that the epidermis of leaf and stem will admit solution and that under certain conditions, absorbtion may ensue through the entire surface. The importance of an examination of the question of absorbtion by Charophyta appears obvious, considering the fact that Bierberg's results have not yet been confirmed.

Experiments with Methylen-Blue.

The first experiment with strongly diluted Methylen-blue solution (1:120.000). Experiment-plant: *Chara fragilis, Ch. foetida, Ch. coronata* and *Nitella mucronata.* Duration of experiment: 24 hours. The experiments were conducted in the following order: 1. plants with rhizoids, 2. plants without rhizoids, 3. plants without rhizoids, but the place of incision was greased with a mixture of lanolin-vaseline.

The following results were derived from these experiments:

1. the plants with rhizoids: — the dye penetrated all the cells from the basis up to the top of the stem;
2. the plants of the second series i. e. without rhizoids: — the dye entered mainly the upper internodial cells but there was less Methylen-blue sediment in the middle and lower parts;
3. the results of the third experimental series were as those of the second but with the least reaction upon the basal internodial cell.

The outcome of the experiment with Chara coronata showed strongest reactions.

Experiment with KNO_3.

Experiments were carried out in the following way: A 7 Centimetres high, 2 Centm. wide testing-tube was used for the experimetal culture. Having planted the specimen in the soil at the bottom of the testing-tube we filled the same up to the brim with 1% KNO_3 solution and stopped it with a cork. We cut the cork in two parts and sank the internodium of the experimental plants into the incision of the cork the outer surface of which we greased with lanolin-vaseline in order to prevent the escape of the KNO_3 solution out of the water. By such experimental arrangement the lower part of the Chara-stem was dipped in the KNO_3-solution, and the upper part in the water. The success of the experiment after 24 hours was so far evident as it proved Diphenylamin and Nitron in the upper part of the stem.

The reverse experiment with H_2O in the testing-tube showed much less reaction — hardly noticeable in the middle of the stem — an evident proof of a much stronger absorption by the rhizoids than by the thallus surface.

These experimental results differ from those of B i e r b e r g in so far only as, according to our own experiments, also the surface of Chara will absorbe some salts and dyes, this being perfectly analogous to all phanerogamous plants.

To confirm these results we made some experiments similar to those by S n e l l in his work on nutrition of submerse water-plants. It simultanously treats the question: do Charophyta get nutritive materials out of the water or from the soil by the action of the rhizoids?

The parallel experiments with plants fixed in the soil and overhanging plants gave the following results.

1. Experiment.

Chara fragilis, 6 plants in each vessel. Duration of experiment 37 days (18. IV.—15. V. 1925.). The numbers in the table show the length of the plants in Centimt. at an average value.

Culture	Hanging			Rooted		
	Beginning	End	Increase	Beginning	End	Increase
Slough	12	19	59%	10.5	40	280%
Pebbly sand . .	—	—	—	10	19	90%

Result of the experiment shows an increase:

a) plants overhanging the slough . . 59%
b) plants rooted in pebbly sand . . . 90%
c) plants rooted in slough 280%

Compare the following fig. 1.

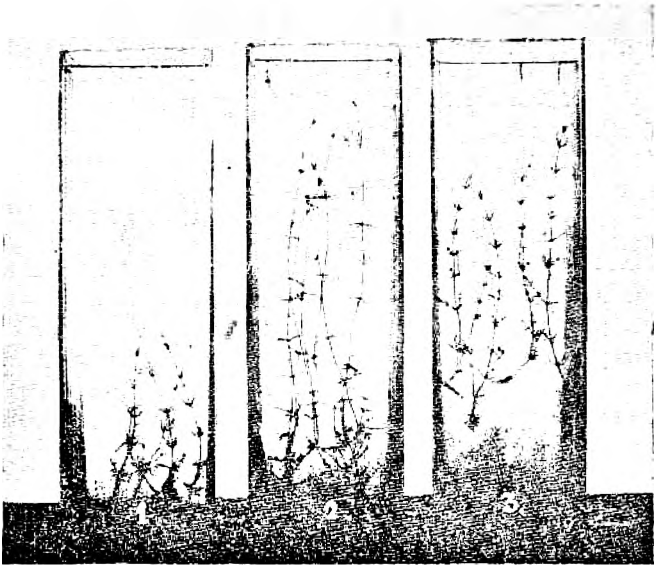

Fig. 1. *Chara fragilis.* 1. rooted in sand. 2. rooted in slough. 3. overhanging slough.

2. Experiment.

Chara foetida, 4 plants in each vessel. Duration of test 24 days (14. V.—7. VI. 1925.).

Culture	Hanging			Rooted		
	Beginning	End	Increase	Beginning	End	Increase
Slough	9.8	20.8	**112%**	6	30.3	**405%**
Clay	10	21.2	**112%**	4.3	20.2	**219%**
Pebbly sand . . .	9.5	22.5	**136%**	4.3	17	**178%**

The result is shown in the table. Plants rooted in the soil generally showed a much richer growth than the overhanging ones. The quality of the soil made no difference.

To test the dependence of the hanging plants from the quality of the soil we made the following experiment.

3. Experiment.

Chara fragilis. Experiment the same as the 2. nd. Duration of test 35 days (20. VI.—25. VII. 1925.); all plants hanging. 6 plants in each vessel.

Culture	Beginning	End	Increase
Slough . . .	7.5	13.6	80·2 %
Clay	7.6	15.1	98.6 %
Sand	8.—	14.5	81.—%
Plain water .	8.—	15.6	95.—%

The results of the experiment showed no great differences in increase of the plants overhanging different soils (Compare fig. 2.).

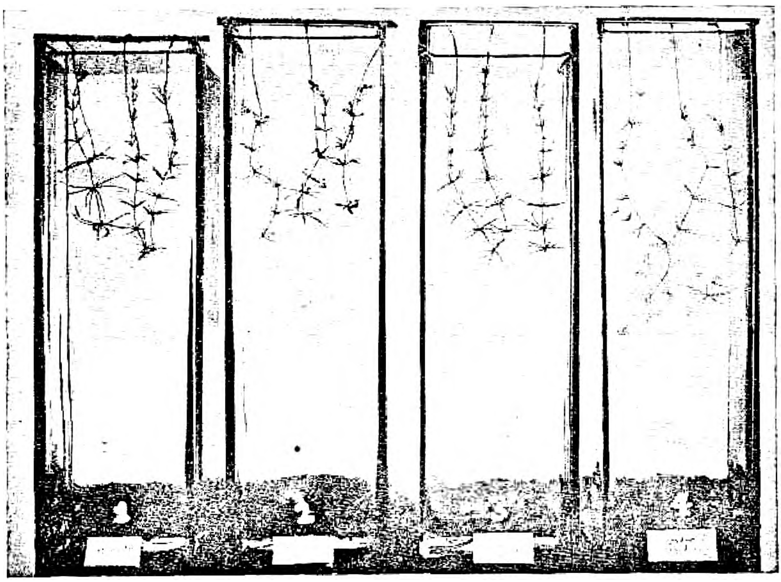

Fig. 2. *Chara fragilis* overhanging 1. slough, 2. clay, 3. sand. 4. plain water.

Pond reached like results in his experiments with phanerogamous waterplants. Bierberg had contrary results i. e. plants rooted in soils rich in nutritive materials grew better than those in washed sand.

Another experiment taught us that the rhizoids are of greater importance for absorption than the stem surface.

4. Experiment.

Chara fragilis: 6 plants to each vessel. Duration of test 20 days. Results:

1. Plants rooted in slough with rhizoids 56%
2. Plants overhanging in slough with rhizoids . . 39%
3. Plants overhanging in slough without rhizoids 21%

Hence the presence of the rhizoids is of special importance for the normal growth of the plants, but particularly so as a condition for absorption.

II. RELATION OF CHAROPHYTA TO DIFFERENT SOILS.

Some investigators have already maintained that Charophyta in culture act differently in different soils. Migula in his systematic treatment of Characeae of Germany (l. c. p. 157.) in connexion with his experiment with *Nitella mucronata* observes as follows:

»Ueberhaupt geben diese Kulturen wohl Veranlassung, auch die Formen anderer Arten in gleicher Weise zu untersuchen und es wird sich dabei gewiss herausstellen, dass man es nicht mit erblichen Eigenschaften, sondern nur mit durch aüssere Verhältnisse bedingten Wuchsformen zu tun hat.«

Ernst, too, has made some occasional experiments. Still extensive experiments in this respect appeared to us worth the trouble.

For these experiments we used first natural soil qualities, such as slough, gardensoil, clay, fine sandy soil, and finally, for comparison, clean washed quartz sand (by Merck). The experiments were carried out under the same conditions of light and temperature.

a) Experiments with Chara fragilis.

In each quality of soil were planted two vessels, each holding 5 stem-ends, of about 7 milims, lengths with 4—5 internodia. Duration of test two months, 26. I.—26. III 1926.

Sort of soil	Beginning	End	Increase in millim.	Increase in %
Slough	6.8	31.3	24.5	346%
Garden soil . . .	6.9	32.4	25.5	369%
Clay	6.1	26.4	20.3	332%
Sand	7.9	29.2	21.3	269%
Quartz	7.7	16.7	9.—	116%

The last figure column clearly shows the result. The constant decrease in growth of the culture from the slough soil down toward the quartz is distinctly striking. Yet the culture of the rich garden mould exceeds that of the slough. But the difference of increase is too insignificant to form a conclusive judgment. In the other cultures the branches too showed decrease in the size of the culture between slough and quartz. The measuring of the length of these twig lengths averaged as follows: slough and garden-soil 3—4 centim., clay 2—5 centim., sand 2—2.5 centim., quartz 1.25 centim. The appearance of the fructification was observed simultaneously. The first oogonia appeared early in February with the cultures in the quartz-sand; a week later they appeared simultaneously in the sand and clay cultures but with the slough and garden soil cultures they appeared only a full week later and only a few in number.

The whole experiment was repeated later on with like results, but with distinctive differences between slough and garden soil. Increase in % amounted to:

a) in slough 395%
b) in garden-soil 279%
c) in clay 304%
d) in sand 179%
e) in quartz-sand 172%

With regard to propagation the results are the same.

b) Experiments with Chara foetida.

Quality of soil	Beginning	End	Increase in Cntm.	Increase in %
Slough	6.3	38.1	31.8	504%
Clay	6.2	19.6	13.4	216%
Sand	6.6	15.4	8.8	133%
Quartz	6.—	12.7	6.7	112%

The results of this experiment are the same as with *Chara fragilis.*

c) Experiments with Chara coronata and Nitella mucronata.

	Chara coronata	Nitella mucronata
Slough	344%	220%
Clay	161%	140%
Sand	77%	60%
Quartz	31%	18%

The subjoined picture plainly illustrates the result (Fig. 3.).

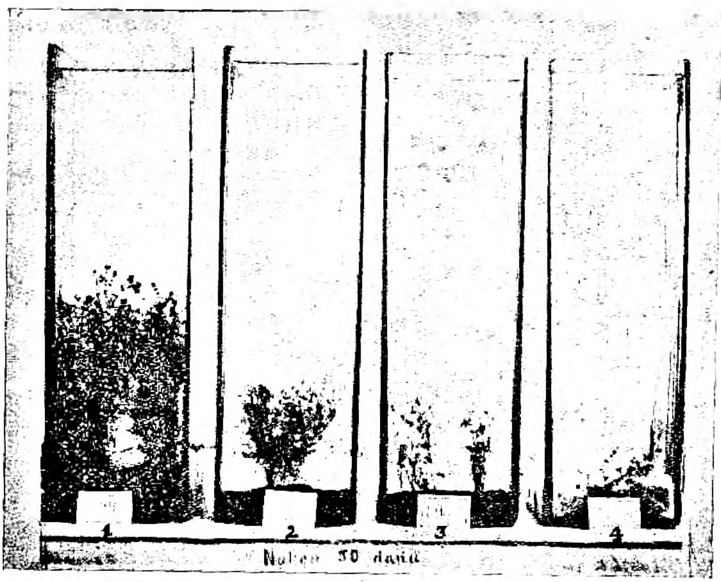

Fig. 3. ***Nitella mucronata*****. Cultivated in different soils;**
1. slough, 2. clay, 3. sand, 4. quartz.

There was a striking difference in the greening of the experimental plants. The slough-cultures were of a rather rich green, while the quartz sand cultures were all faded, possibly as result of insufficient nourishment. Finally, there was a marked difference with respect to lime incrustation especially between the sand and clay cultures and the slough cultures respectively. The plants of the sand- and clay-cultures were much stronger and incrusted.

Summarising all the results we obtained from the experiments with various species of Characeae, we can say as follows:

1. Characeae grow best and most abundantly in natural slough; but their growth markedly decreases in garden, clay and sandy soil.
2. The weaker the nutrition, the earlier the fructification.

Regarding the fact that Characeae grow best in slough-soil, we should like to learn the cause of this phenomenon. In the first place the different organic substances as well as pH concentration could be held responsible for it. The presence of rich mikroflora in the slough-ground is, at all events, of some influence, because some experiments with sterilised slough, showed a decrease of 50%. Also the CO_2 fertilisation of the slough-soil and an ammoniac-formation might be taken as influencing factors. All these suggestions are left to further investigations.

III. THE INFLUENCE OF LIGHT.

It is known from observation in nature that the Charophyta prefer shady places. Some Characeae at Geneva-Sea and Vierwaldstätter-Sea (Forel, Chodat), reach a depth of 8—10 metres Some species of Nitella penetrate to a depth of 30 metres. We find, nevertheless, Characeae also at the open border of quiet waters often directly exposed to the effect of sun. Regarding the conditions of light no experiments have as yet been made — to our knowledge at least — except those by Karling which prompted us to try to orientate ourselves on this question.

Experiments were made with *Chara fragilis, Ch. foetida, Ch. coronata* and *Nitella coronata*. It was not our intention to determine the actual »Lichtgenuss«. We primarily only wished to watch the vegetation growt and fructification of Characeae in places of differing intensity of light.

The first series was exposed directly to the sun, the second to diffuse light, and the third to a weakly diffuse light. Thus the intensity of light decreasing from sun, to shade, and deeper shade respectively, was about 1 : $^1/_{10}$: $^1/_{1000}$. Parallel experiments in the dark were also made.

Experiments with Chara fragilis.

The culture in slough with 4 plants in each vessel. Duration of test 46 days (21. IV.—7. VI. 1925.).

The shade-cultures in diffused light showed best growth, not only in length, but also in the number of internodia and in the length of their leaflets. The latter were twice as long in the shade-cultures as in the light-cultures, which were paler and in general wore a sickly aspect. It is important to emphasize that with light-cultures the fructification sets in much earlier and richer than with the shade-cultures. It is a striking fact that the culture in dark, though without light, showed remarkable growth nevertheless.

	Beginning	End	Increase
Sun	12	25	108%
Shade	11.50	77	567%
Deep shade	17.7	33	180%
Darkness	12	17.5	45%

Most of the experiments with *Chara foetida, Ch. coronata, Nitella mucronata* gave similar results:

	Direct sun-light	Shade-diffused light
Chara fragilis . . .	60%	400%
Chara coronata	80%	344%
Nitella mucronata	40 %	320%

The cultures in the sun showed a smaller growth in length, a smaller formation of chlorophyll, more incrustation and earlier fructification.

The cultures in shade showed an intensive growth in length, a strong chlorophyll formation, weak incrustation and a late fructification.

One more experiment should not be omitted in which both the soil agency, and the light agency were changed.

Chara fragilis: 6 plants to every vessel; duration of test 2 months.

		Beginning	End	Increase
Slough . . .	Sun . .	6.8	23.3	242%
	Shade . .	11.3	52.—	360%
Pebbly sand .	Sun . . .	7.—	11.6	66 %
	Shade . .	13.—	28	115%

Comparing the above experiments it should be considered that from te beginning the experimental plants were kept a little longer in the shade than were those in the light. It can be seen from the experiment that the increase of the shade plants is in both cases greater than the one of light-plants. The light-factor appears of much more importance than that of the soil. Fructification ensues earliest with the light-sand cultures, which agrees with Klebs's known rule i. e. that weak nutrition and insolation hasten the fruit formation. Karling obtained similar results. He says: »Under the condition of the experiments described, light of day appears to be a primary factor in inducing the formation of antheridia and oogonia«.

IV. SOME EXPERIMENTS WITH NUTRITIVE SOLUTIONS.

The experiments to cultivate the Characeae in artificial solutions to prove the necessity of certain elements were but occasionally made. Nonweiler complained of the failure of his experiment to cultivate *Chara strigosa* in nutritive solution. He communicates that his experimental plants in the artificial solution perished within a short time. We had the same experience with our experiment of *Chara fragilis* in the known nutritive solutions of Knop, Molisch, Artari and v. Crone. Better results were obtained with about $^1/_{10}$ of normal-solutions to which a sprinkling of Calciumsilicat was

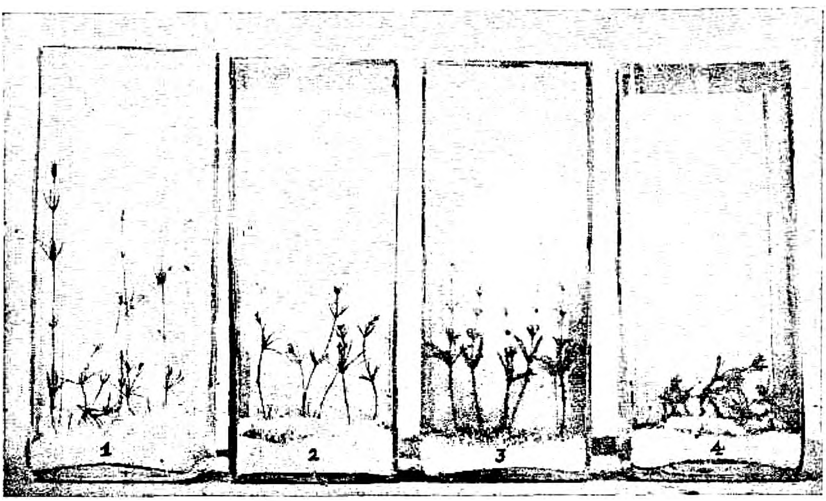

Fig. 4. *Chara fragilis,* cultivated in nutritive solutions, 1. clean water, 2. normal solution, 3. solution with Ca $(HCO_3)_2$, 4. Ca-free solution.

added. The same improved result we obtained from Ca/HCO₃/₂. Not in one single case did we observe such an abundant growth as with the slough cultures. We therefore regard all these experiments as merely preliminary ones whose negative results may serve as a guide for further research which must be conducted with more exact methods, under consideration of all nutritive factors and especially pH. We should not have published our results if we were not in position to prove by our experiments that Calcium is indispensable for the nutrition of Characeae.

The outcome of this experiment with *Chara fragilis* was as follows (Fig. 4.):

in normal solution	increase	26%
in Ca-free normal-solution	«	0%
in solution with $Ca/HCO_3/_2$	«	68%
in clean water	«	152%

There was no growth at all in the Ca-free solution. Moreover, the plants were of a sickly aspect and finally died. The above figures show it clearly. Although the nutritive solutions were not of the best for the development of the plants, the Calcium was obviously indispensable for their growth. The Charophyta, regarding their want of Calcium, may naturally be counted to the group of »higher« algae which in accordance with Pringsheim cannot exist without Calcium.

Summary.

1. The rhizoids represent the main organs of absorption of nutritive materials by Charophyta. The stem i. e. thallus surface has in this respect but a subordinate function. They can therefore as far as absorption is concerned be compared to the phanerogamous submerse water-plants.

2. The Charophyta grow best in natural slough soil, and in marked decrease steadily weaker in garden-soil, clay- and sandy-soil.

3. The Charophyta are typical shade-plants because they grow best in diffused shadowy light. Their vegetative growth is strongly influenced by direct light.

4. Weak nutrition and strong insolation hasten the fruit formation of the Charophyta.

5. Calcium is indispensable to *Chara fragilis*.

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