Some Methods for Use in the Culture of Algae[1].

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With Plate XXVIII.

THE following notes are somewhat of the nature of suggestions, since much has still to be done, no doubt, before the efficacy of the treatment and the faults and difficulties of the methods in detail are fully demonstrated; but since the author has found they can be used with some measure of success, the various workers interested in the culture of Algae may care to take the methods up and try to improve them.

1. If agar is swollen in dilute acetic acid, and then washed very thoroughly so that every trace of soluble salt is removed, it can be used, mixed with the necessary culture fluids, as a convenient medium for the growth of some Algae, as Beyjerinck had already observed. But, so far as the author knows, the use of such a medium for separating Algae in plate culture and for observing their growth in hanging drops has not been attempted. It can be done, however, though the high melting-point of the agar and the sliminess of some Algae occasionally cause difficulties.

The author has also succeeded in separating Algae by the following methods:—

2. Shake them up in sterilized nutritive mineral solution, mix rapidly with silica jelly, also sterilized, and pour into

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glass dishes. With species of *Oscillaria* and of *Palmella*, certain Protococcoideae, &c., the author has observed growth in hanging drops of this silica-jelly medium under high powers, and has seen sufficient to make it hopeful that even Diatoms may be cultivated this way; and it is not impossible that some modification of the process could be utilized for the culture of marine Algae.

Another device is as follows:—

3. Shake the Algae up in the nutritive solution and rapidly mix with sterilized plaster of Paris, and pour into dishes. The fixed Algae grow *in situ* in some cases, but others appear to be too sensitive for such treatment.

Experiments have also been made as follows, with some success:—

4. The Algae are shaken up in the culture medium, and a large quantity of lime-water quickly added. Then carbon dioxide gas is passed rapidly through, and the Algae are thrown down with the precipitate of calcium carbonate; this is poured into dishes as if it were plaster of Paris. Perhaps this method could be utilized in the study of calcareous Algae, but with some forms it appears too drastic. Baryta seems to act as a poison, and cannot be substituted for lime.

It is clear that if once we obtain a pure colony on a glass dish, a trace fished out with a needle may be used to start other cultures. Season, temperature, intensity of light and other factors, are of importance in these matters.

As an illustration of what may be done with the hanging drop culture I take the following example:—

On August 21 a quantity of *Oscillaria—O. tenerrima* (Ktz.) apparently—was shaken up in Knop's mineral solution, added to silica jelly and poured with a drop of sodium chloride into a plate, where it soon set. On the 22nd I made a hanging drop of a small portion of this, and fixed a small piece of the Alga under a 4 mm. dry apochromatic lens with No. 2 eye-piece with micrometer. The piece measured 13·5 μ long × 2·25 μ broad, and next day showed no perceptible change. Nor could I detect any growth on the 24th, or

following days; but by the 25th it was evident that growth was taking place in other pieces scattered over the field, and on the 26th I fixed another segment, measuring 45 μ long × 2·3 μ broad, and obtained the following measurements. See Plate XXVIII.

Date.	Hour.	Temp.	Length.	Elongn.
Aug. 26.	11.30 a.m.	22° C.	45 μ	
,, 27.	3.10 p.m.	25	58·5 μ	13·5 μ
,, 28.	10.20 a.m.	21·5	68·4 μ	9·9 μ
,, ,,	12.50 p.m.	21·5	72·9 μ	4·5 μ
,, ,,	3.30 ,,	22	76·5 μ	3·6 μ
,, ,,	6.15 ,,	21·5	78·75 μ	2·25 μ
,, 29.	10.0 a.m.	18·5	85·5 μ	6·75 μ
,, ,,	12 noon	21	90·45 μ	4·95 μ
,, ,,	3.35 p.m.	21	99·0 μ	8·55 μ
,, ,,	8.0 ,,	20·5	105·75 μ	6·75 μ
,, 30.	9.0 a.m.	19·5	109·0 μ	3·25 μ
,, ,,	1.15 p.m.	23	113·5 μ	4·5 μ
,, ,,	3.30 ,,	23	123·5 μ	10·0 μ
,, ,,	6.45 ,,	22	132·75 μ	9·25 μ
,, 31.	9.0 a.m.	18·5	137·25 μ	4·5 μ
,, ,,	12 noon	20	148·5 μ	11·25 μ
,, ,,	3.30 p.m.	21	162·0 μ	13·9 μ
,, ,,	6.50 ,,	20	171·0 μ	9·0 μ
Sept. 1.	9.15 a.m.	17	175·5 μ	4·5 μ
,, ,,	12.30 p.m.	19·5	189 μ	13·5 μ
,, ,,	3.5 ,,	20	198 μ	9 μ
,, ,,	6.40 ,,	21	207 μ	9 μ
,, ,,	9.45 ,,	20	211·5 μ	4·5 μ
,, 2.	5.25 a.m.	17·5	211·5 μ	0
,, ,,	10.15 ,,	18	213 75 μ	2·25 μ
,, ,,	1.10 p.m.	20	216·0 μ	2·25 μ
,, 3.	10 0 a.m.	16·5	235·0 μ	19 μ

From the fact that this filament was held by the tail, as it were, I could not determine whether one only or both ends were growing.

It took from 11.30 a.m. on Aug. 26 to the same hour on Aug. 29 (i. e. 72 hours) to double its length—from 45 to 90 μ—and this appears to be very slow growth, though I am not aware that measurements exist to help us in deciding *how* slow.

The next doubling of the length—90–180 μ—was completed about 10 a.m. on Sept. 1—i.e. in about 70½ hours.

In a diagram made at the time (Plate XXVIII), the curve of growth shows very clearly that no elongation took place during the hours of darkness, but that growth and assimilation are coincident, a point which appears worthy of note in view of what we know as to the effect of light on growing organs.

It may also be added that I have been able to observe the division into zoospores of certain Protococcoideae in hanging drops of agar, and that this appears to occur only during the night.

In connexion with the action of light on green Algae, experiments in which ordinary (reflected) light was allowed to act on agar plates of Protococcoideae, covered with a stencil letter, showed its effect in a few days; the Algae in the area exposed to light developed normally, but those in the non-illuminated parts remained undeveloped. Consequently a faint green letter appeared in a colourless ground.

When the light was more intense, however, the exposed Algae were killed, whereas those in the covered parts of the agar developed fairly well, and thus the surprising phenomenon of a colourless letter in a green matrix appeared. The development in the shaded parts was probably due to the reflection of some of the intense light at the back of the plate, and this diffused over the non-exposed area was strong enough to enable the Algae to grow, but not sufficiently intense to kill them.

Many questions involving the use of quartz and coloured screens, and of different sources and intensities of light arise out of these experiments, and are held over for further investigation.

EXPLANATION OF PLATE XXVIII.

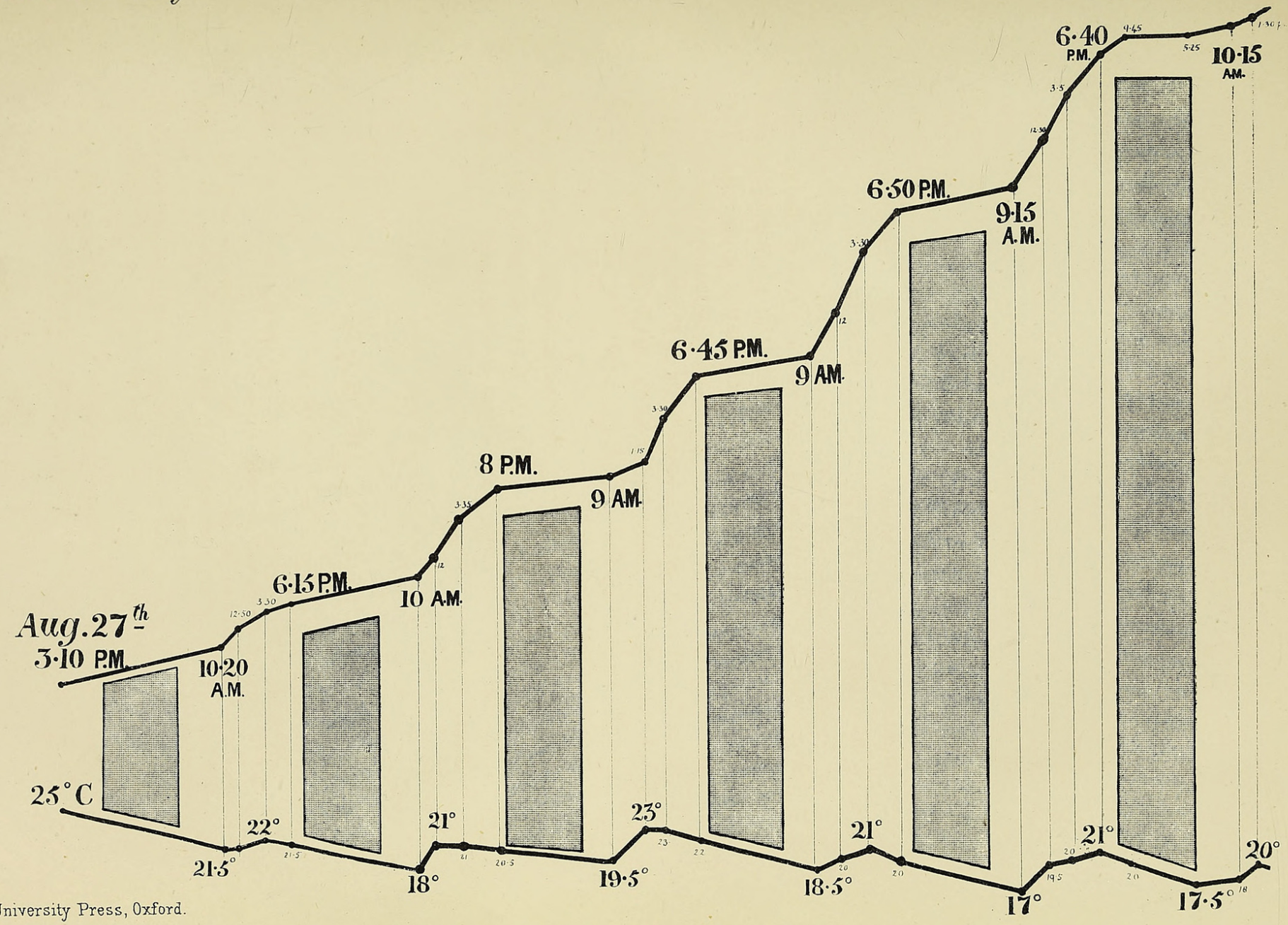
Illustrating Prof. Ward's paper on the Culture of Algae.

The upper curve represents the growth of the filament referred to on p. 565, and the lower curve the changes of temperature.

The curves start at 3.10 p.m. on Aug. 27, when, as seen from the table (p. 565), the filament measured 58·5 μ in length. The shaded areas represent the hours of darkness of each successive night.

It will be seen that the period of maximum growth coincides with that during which assimilation is active—i. e. from 9-10 a. m. to 6-7 p. m.—and that little or no growth occurs at night.

On comparing the curve of temperature we see that it does not explain the ups and downs of the upper curve; maximum growth occurs with falling temperature.



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