ON WATER EXOSMOSIS IN CHAETOMORPHA LINUM (MÜLL.) KÜTZ.

by

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INTRODUCTION.

§ 1. *Statement of the problem.*

The water economy of a plant cell is a function of metabolic relations both in the internal- and in the external milieu.

The outer environment either contributes water to- or removes water from the cell; which cell may either produce or consume water in its metabolic processes. Under water economy we should understand, therefore, a dynamic condition. A continuous intake and production of water, at least in the living green cell, must take place.

The present paper is confined to the discussion of the removal of water from certain cells by water-attracting solutions. This removal of water may be the cause of a great many curious phenomena in the cell, phenomena still imperfectly understood. Of these phenomena chiefly three have received more attention, to wit: plasmolysis, membrane swelling, and protoplasmic swelling.

Plasmolysis depends, in the first place, upon the presence of a vacuole and, moreover, upon the consistency of the protoplasm, the adherence of protoplasm to the wall, and also upon the consistency of this wall.

Supposing a cell with a perfectly elastic wall surrounding a thin layer of protoplasm within which a large vacuole is present, removal of water from such a cell will cause the wall to assume a length corresponding to its unstretched condition. Further removal of water will cause the protoplasm (which is supposed here not to adhere to the wall) to recede from this wall and to surround an ellipsoidal vacuole. Still further removal of water will cause increasing shrinkage of the ellipsoid.

In this process we also assume the cell, at the outset, to be saturated with water; excluding, in this way the so-called suction-pressure (often erroneously called suction-force, see Ursprung and Blum 1916, Oppenheimer 1930, Pringsheim 1931, Ernest 1931, 1934).

In the process, as described above, the volume of the cell will change until the wall is no longer stretched, the volume of protoplast and vacuole will diminish on further loss of water. However, if the turgor-pressure of the protoplasm against the wall may have been sufficiently large, removal of this pressure may cause

a new equilibrium to be set up between the wall and the surrounding liquid, causing the wall to swell.

This phenomenon may be counteracted by increasing the water-attractive power of the surrounding liquid. A similar swelling-phenomenon may be observed in the protoplasm, but only if vacuoles are absent or the central vacuole is small. By selecting an object for the study of water-exosmosis it appears from the above that properties of vacuole, protoplasm and wall determine to a large degree the nature of the phenomena to be observed.

a. If the vacuole be large, the protoplast thin and the wall thin and rather elastic we meet with the classical case as studied by Hugo de Vries (1877); the water neither affecting wall nor protoplast.

b. If the vacuole be large, the protoplast thin but the wall heavy and subject to swelling and shrinking, the exosmosis of water will also affect the wall.

c. If both protoplasm and 'gelatinous' wall are present, exosmosis of water will affect visibly both protoplasm and wall.

The object selected by us, Chaetomorpha Linum, belongs to class *b*. The phenomena of plasmolysis are complicated by the behaviour of the wall.

The question arose whether the role of vacuole, protoplasm and cell-wall in water-exosmosis could be determined separately in this case. As the plasmatic layer is very thin, only the vacuole and the wall remain as important factors.

§ 2. *Discussion of the methods used.*

1. 50 % Plasmolysis.

For the study of plasmolytic phenomena in a quantitative way the well-known method developed by Hugo de Vries (1877) forms the basis of all further work. The concentration of the cell sap was determined by de Vries by measuring the concentration of the external solution in which 50 % of the cells showed incipient plasmolysis. This method should, therefore, be considered as statistical.

2. Plasmolysis-time.

By plotting the time necessary for incipient plasmolysis against the concentration of the external solution, a curve is obtained which is asymptotic at a concentration to be considered as isotonic with that of the cell-sap. A method for the determination of vacuolar concentrations based upon this 'plasmolysis-time' we owe to F. Weber (1929). Prud'homme van Reine has applied

this method in his thesis (1935) on the influence of temperature upon protoplasmatic consistency.

3. Plasmometry.

Measurements of vacuole-volume were carried out by Höfler (1918) the cells being immersed in hypertonic solutions. Knowing the volume of the vacuole before plasmolysis, the relation

$$O_u : O_p = V_p : V_u,$$

should be fulfilled, in which

O_u = osmotic value of the cell-sap in the unplasmolyzed cell (to be determined).

O_p = osmotic value of the hypertonic solution.

V_p and V_u being the volumes corresponding to O_p and O_u respectively.

This simple application of Van 't Hoff's solution law may only be made successfully if the volumes may be determined accurately and if, moreover, the protoplasmic factor does not influence the results.

4. Microcolorimetry.

A modification of Höfler's method in which the (often very inaccurate) measurement of vacuole-volume is unnecessary, was developed by D. J. Kuenen and myself (1935). The anthocyanins in the vacuoles do not leave this vacuole on plasmolysis. As anthocyanin solutions follow the law of Beer-Lambert, every decrease in volume of the vacuole is concomitant with an increase in intensity of the red colour. The intensity of this colour could be measured by means of an especially designed microcolorimeter and, after the thickness of the coloured vacuole was measured, the law of Van 't Hoff was found to apply to the exosmosis of water.

5. Chemical analysis.

There are several direct methods, of which the direct chemical analysis of the vacuole-sap (Wodehouse 1917, Osterhout 1922, Hoagland & Davis 1923, Collander 1930) is only applicable, however, to very large cells. It cannot be denied that this method may yield more information than all of the others put together.

6. Cryoscopy.

Determinations of the freezing point-depression of plant juices which may yield (with several restrictions) direct osmotic values of

the vacuole sap were widely applied by Walter and his pupils (Walter 1928, 1930, 1931*b*, Oppenheimer 1932).

We feel, however, that, like the direct chemical method, this method may be applied only to certain plants or plant cells. If the wall be susceptible to swelling, like in the Fucaceae (Zaneveld 1937) or in the Rhodophyceae (Walter 1923) the extraction of the juices by pressure may yield unreliable results.

7. Refractometry.

If water be excreted in hypertonic solutions, these solutions may become measurably diluted. This dilution may be measured by means of a refractometer. The method is developed further in this thesis.

8. Voluminometry.

The shrinkage of the entire cell may be measured in a simple dilatometer. This method is also dealt with later in this paper.

9. Other methods.

Methods in which either strips of tissue are used (De Vries 1884, Ursprung & Blum 1916, Ernest 1931, 1934, Hoffmann 1932) or in which the so-called 'suction-force' is ascertained shall not be described in this paper, as they do not pertain to the case dealt with.

CHAPTER I.

DESCRIPTION OF THE ALGA.

Chaetomorpha Linum (Müll.) Kütz. consists of long free-swimming filaments. The cells are cylindrical, their diameter amounts to 300—500 μ. The length of the cells is rather variable. The young cells are nearly square, the old ones twice as long as broad.

Intercalary longitudinal growth is apparent.

The thickness of the cell-wall in living cells is $\pm$ 4 μ; in cultures which have been kept for some weeks in the laboratory, the walls become heavier; 8—9 μ. There are many chloroplasts which are pressed against the cell-wall so that it is almost impossible to see this wall in living filaments.

§ 1. *Literature.*

a. The cell-wall.

Correns (1893) already observed in a comprehensive study the structure of the Chaetomorpha-membrane. The longitudinal wall consists of an inner- and an outer layer. Each of those two layers consists of numerous "lamellae". When the contents of the cell are removed with the help of "Eau de Javelle", the filaments show a very fine, longitudinal and transversal striation. There are, therefore, two systems of striations, the one a laevogyric spiral with a speed of 20—30° with the axis of the filament, the other one, nearly perpendicular to the first, is dextrogyric. The lines differ in two respects from the striation of the walls in the cortical fibres of Vinca for in the alga the striae are not always rectilinear, but often waved and irregular.

As the result of careful observation Correns concludes;

1. the striations of the algal membrane is caused by a fine corrugation of the lamellae.
2. each lamella is only ribbed in one direction.
3. the direction of the ribs changes in subsequent lamellae of the same membrane. Usually the ribs of the second lamella are perpendicular to those of the first and the third, and parallel to those of the fourth lamella.

4. the lamella are parallel to the direction of the ribs, differentiated in striations of different texture.
5. each lamella shows two layers; the first one compact, the second loosely built. This lamellation is visible due to difference in water content.

Nicolai and Frey-Wyssling (1938) studied the submicroscopical structure of the cell-wall of Chaetomorpha. They also assume two layers; an inner and an outer one. The outer layer encloses the whole filament, the inner one surrounds the single cells.

Staining shows no difference in the composition of the two layers, both swell strongly in various solutions, and this swelling makes it possible to observe the separate lamellae in the layers. The inner layer swells much more than the outer one, the outer one is much more compact. The lamellae of the inner layer protrude by swelling into the lumen of the cell, with a wavy contour. Correns described the striation in Chaetomorpha melagonium (Web. et Mohr) Kütz. and found a repeated alternation in the direction of the striae.

In their study of Chaetomorpha Linum the authors found only one single longitudinal and one single transversal system, not only in the intact filaments, but also in longitudinal sections of the filaments.

The number of lamellae is only approximately indicated as fifteen. The outer lamellae are firmly attached to one another and it is difficult to distinguish them optically.

The authors also studied the structure of the cell-wall by means of the polarization-microscope, and they endeavoured to elucidate the submicroscopic structure by a combination of the optical and the microscopic data. The Chaetomorpha-filament as a whole appears to have a tube-structure ("Röhrenstruktur", Frey-Wyssling, 1935).

From the optical, chemical and X-ray data Nicolai and Frey-Wyssling concluded that the cross-striation should not be attributed to differences in position of subsequent lamellae, but that this striation is inherent to a single lamella. The striation system is, therefore, submicroscopic and each lamella contains both the longitudinal and the transversal system.

b. Plasmolysis in Chaetomorpha.

Several authors have already studied the plasmolysis-phenomena in Chaetomorpha Linum.

Kotte (1914) pointed out that for the greater part of the marine algae studied by him, amongst which Chaetomorpha Linum, an

exact determination of the osmotic value of the cell-sap was totally impossible. This failure he ascribes, in the main, to three factors;
1. the swelling of the membrane
2. the permeability of the protoplasm
3. the elastic contraction of the cell-wall in plasmolysis.

It is evident that these three factors act in the same direction. The swelling of the membrane is always directed towards the lumen of the cell, the elastic contraction of the membrane diminishes the volume of the cell.

Plasmolysis becomes only visible after significant concentration of the cell sap, and corresponding to this an increase of the osmotic value.

Penetration of the plasmolyte may also cause an exaggerated reading of the osmotic value.

In the first part of his publication Kotte investigates the influence of different plasmolytes on each of the three above-mentioned factors in several algae. In the second part of his work he studies the swelling of the Chaetomorpha membrane in a great variety of solutions. Unfortunately he prepared these solutions by dissolving the salts in distilled water, ignoring by this the balanced action of the salts dissolved in seawater. He finds a correspondence between the influence of the anions upon the swelling and the Hofmeister series;

$SO_4 <$ acetate $< Cl < NO_3$ and Hofmeister;
$SO_4 <$ tartrate $<$ acetate $< Cl < NO_3 < Br.$

The H-ions promote the swelling most markedly, the OH-ions, on the contrary, prevent the swelling of the Chaetomorpha-membrane.

Often a parallelism exists between the influence upon the swelling and the toxic action of several solutions.

The kations increase the swelling velocity in the following sequence;

$$Ca = Ba = Sr < Mg < Li < K < Na < NH_4 < H.$$

In alkaline solutions there is no swelling. In neutral salt solutions the swelling velocity diminishes with increasing concentration.

In solutions of mixed salts the swelling velocity is an average of the velocities of the one-salt solutions, in some cases there appeared some antagonistic action of the components in the salt solution, so that the observed swelling velocity is less than that in each of the one-salt solutions.

Höfler (1932) describes the forms of plasmolysis of Chaetomorpha. In his publication some photographs illustrate, better than a description could do, the several irregular forms that are found in plasmolysis of Chaetomorpha Linum.

In a concentrated hypertonic plasmolyte, containing 2.5 gr M. ureum the longitudinal walls and their surfaces form deep hollows. The protoplasts remain attached to the inner part of the transverse walls.

The Chaetomorpha filament showed positive 'plasmolysis spots' at the longitudinal walls, negative 'spots' on the transverse walls (terminology of Weber 1929). The younger cells that are more or less square, often show another distribution of these 'plasmolysis spots'. This is demonstrated in fig. 8a and b of Höfler's publication. On the transverse wall there are as many positive 'plasmolysis spots' as on the longitudinal walls. Relatively deep, round pits occur. Sometimes the receded protoplast obtains the form of a diabolo. From Höfler's publication it appears that, in plasmolysis, the vacuoles have an all but regular form.

Hoffmann (1932a) cites the work of Kotte, who also concluded that the concentration of the incipient contraction approximates the osmotic concentration of the cell sap. Concomitant with this contraction, however, the membrane begins to swell, and this creates another uncertainty in the results.

With this conclusion of Kotte, Hoffmann does not agree, the swelling forces are always acting towards the centre of the cells, here with influencing only the degree of contraction and not the moment of its inception. So he applies his modified method on Chaetomorpha and determines the incipient contraction and the incipient swelling. He compares the values obtained in this way with the values of incipient plasmolysis.

Later on (1932b) Hoffmann concludes that this comparison is not justified as he did not determine the osmotic value in the case of turgescence but the suction pressure in the case of incipient swelling or incipient contraction. In 1935 he showed that a number of marine algae have no active suction pressure, so that the values found by him in his first publication, are not real. The difficulties to apply the method of Höfler in the case of Chaetomorpha are pointed out by Hoffmann once again in 1936.

Only by means of the method modified by Prát (1922) it was possible to determine the degree of plasmolysis in solutions of saccharose, glucose and glycerol (1 M.), but only very inaccurately. Prát observed the retrogression of the plasmolysis by drawing the different stadia or following the phenomenon on moving films.

§ 2. *Culture.*

We received our material from the Zoological Station, Den Helder. The material was shipped dry, in a closed box. In the laboratory we kept the material in large cristallization-dishes (diameter ± 20 cm), covered with glass plates, to prevent the evaporation and to protect against rainfall, for during the greater part of the summer the dishes were placed on a balcony at the North-side of the laboratory. In winter the dishes were placed in one of the greenhouses, to prevent freezing. The dishes were filled up for about one half with artificial seawater and in each dish was placed a small quantity of filaments, so that exchange of gas is not impeded. In this way we had at our disposal, practically during the entire year, fresh material in good condition. We received fresh material about monthly from Den Helder [1].

The artificial seawater was prepared after the "Challenger-recipe" with some modification;

NaCl	27.21	gr./L. aq. dest.
$CaCl_2$	1.26	gr.
$MgCl_2$	3.80	gr.
$MgSO_4$	1.66	gr.
K_2SO_4	0.863	gr.
Na_2CO_3	0.67	gr.
$MgBr_2$	0.076	gr.

To each Liter solution 0.714 gr. of the modified Kolkwitz mixture is added (KNO_3 20 gr., KH_2PO_4 5 gr., $FeCl_3$ 0.1 gr.). Moreover, the artificial sea-water contains 100 cc soil-extract/Liter, obtained by boiling a quantity of gardensoil in twice its quantity of water for an hour, afterwards filtered off.

Usually the addition of KH_2PO_4 and soil-extract causes the formation of precipitate. After settling for one night the precipitate could be filtered off.

The refractive index, controlled with a refractometer after A b b e proved to be $N_D^{20} = 1.3389$ and the total salt-concentration 3.6 %. To obtain the refractive index and the concentration of the natural seawater (3.2 %) 125 cc aq. dest. were added per Liter solution. The refractive index of the artificial seawater prepared in this way is $N_D^{20} = 1.3381$ and the pH 8.6 (natural seawater $N_D^{20} = 1.3386$, pH 8.15).

Weekly the refractive index of the liquid in the dishes was

[1] At this place I wish to thank Dr J. V e r w e y, director of the Zoological Station at Den Helder for his kind help and continuous care.

controlled and evaporation was belanced by addition of the required quantity of distilled water.

§ 3. *The environment of Chaetomorpha Linum.*

When the material is fresh, it is crisp and tough, whilst after some weeks it becomes flaccid and more or less slimy. For our experiments we used fresh filaments exclusively.

It is a well-known fact that the influence of one salt may be neutralized partly or totally by another.

After Sydney Ringer's (1884) discovery and its amplification by Loeb (1908), we realize that antagonism is of the greatest importance, especially for marine organisms. For in the milieu of these organisms the relations between the different salts may vary considerably.

The environment of one of these salt organisms, the filamentous green alga Lochmiopsis was studied by Ruinen (1933). The natural environment of this alga is subject to considerable variations, both in temperature as in the chemical composition of the brine in which the algae are found. This alga seems "to be particularly adapted to the sudden changes which may occur in its environment. Moreover, it is most probable that in a milieu variable in composition to such extent, an alga may present itself under different forms. And many investigators have shown how trivial changes in the medium exert a prominent change in the morphological characters".

In the laboratory the pH-, temperature- and salt-influences were studied and for those three factors the "potential milieu" was delimited. The "potential milieu" is defined by Baas Becking (1931) as the sum total of the factors, chemical as well as physical, under which an organism may occur. The natural environment is the sum total of the factors under which an organism does occur naturally. It is self-evident that the natural milieu is more restricted than the potential and for Lochmiopsis the natural environment lies also well within the potential milieu.

In comparing the studies of Jacobi and Baas Becking (1933) on Artemia salina (L.), Hof and Frémy (1933) on blue-green algae, Petter (1932) on red salt-bacteria and the data available for Dunaliella viridis Teod., Ruinen concludes that "the potential milieu of all these forms is different and also differs from that of Lochmiopsis".

In reproducing the potential environment in several more salt-solutions, Ruinen uses the so-called triangular-diagrams, the well-known method in physical chemistry, applied by Baas

Becking for this work. This method is also applied in the determination of the "potential milieu" of Chaetomorpha in triple-salt combinations of NaCl, $CaCl_2$ and $MgCl_2$. Each point of the triangle corresponds to a definite relationship of the ions Na^+, Ca^{++} and Mg^{++}, expressed in equivalent concentrations. In this way it is possible to indicate the combinations that are non-poisonous for the organism studied.

To study the potential milieu of Chaetomorpha the behaviour of some filaments is observed in a large series of flasks containing several concentrations of 21 different triple-salt combinations.

2 N NaCl, 2 N $CaCl_2$ and 2 N $MgCl_2$ solutions (the Merck-salts "pro analyse" are used) in distilled water are prepared as stock solutions. From these stock solutions all required combinations are prepared by mixing and diluting. Moreover, each Liter solution contains 100 cc soil extract and:

0.3 gr. K_2SO_4
0.15 gr. KH_2PO_4
0.6 gr. KNO_3.

This is also added to the diluting liquid, so that in each flask the same quantity of these indispensable nutrient salts is present.

We used 10 series, each consisting of 21 different combinations. Each 50 cc flask contained 30 cc salt solution.

The total concentration of these 10 series is:

I	0.05 Eq.
II	0.10 ,,
III	0.30 ,,
IV	0.50 ,,
V	0.70 ,,
VI	0.90 ,,
VII	1.00 ,,
VIII	1.25 ,,
IX	1.75 ,,
X	2.00 ,,

Each series is composed of 21 different ternary combinations; NaCl + $CaCl_2$ + $MgCl_2$, corresponding to progressive differences in the components of 20 % intervals.

These components are placed in the triangular diagram fig. 1.

Each of the ten series may be represented by such a diagram.

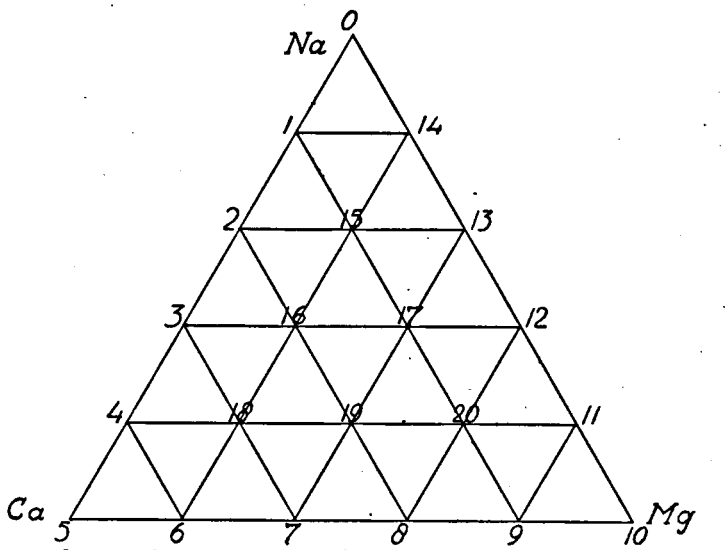
After the flasks are filled, some short Chaetomorpha filaments are

brought into each flask. The flasks are stoppered with a cotton plug and placed in a North-window of the laboratory [1].

After three days the condition of the filaments was observed macroscopically.

Figure 2 shows the results obtained.

The figures are indicating the condition;



The numbers refer to the numbers of the flasks in each series

Fig. 1.

5: normal, or nearly normal filaments.
4: the filament is somewhat shrunk or somewhat rosary-shaped or less green than in the first case.
3: the filament is partly green and normal, partly already dead.
2: part of the filament is dead, another part in condition 4.
1: the whole filament is strongly rosary-shaped and brownish, or no longer green.
0: the filament is dead.

These figures are plotted in the triangular diagrams (fig. 2) and

[1] At this place I wish to thank Mr R. B o o m for help in the preparation of the solutions.

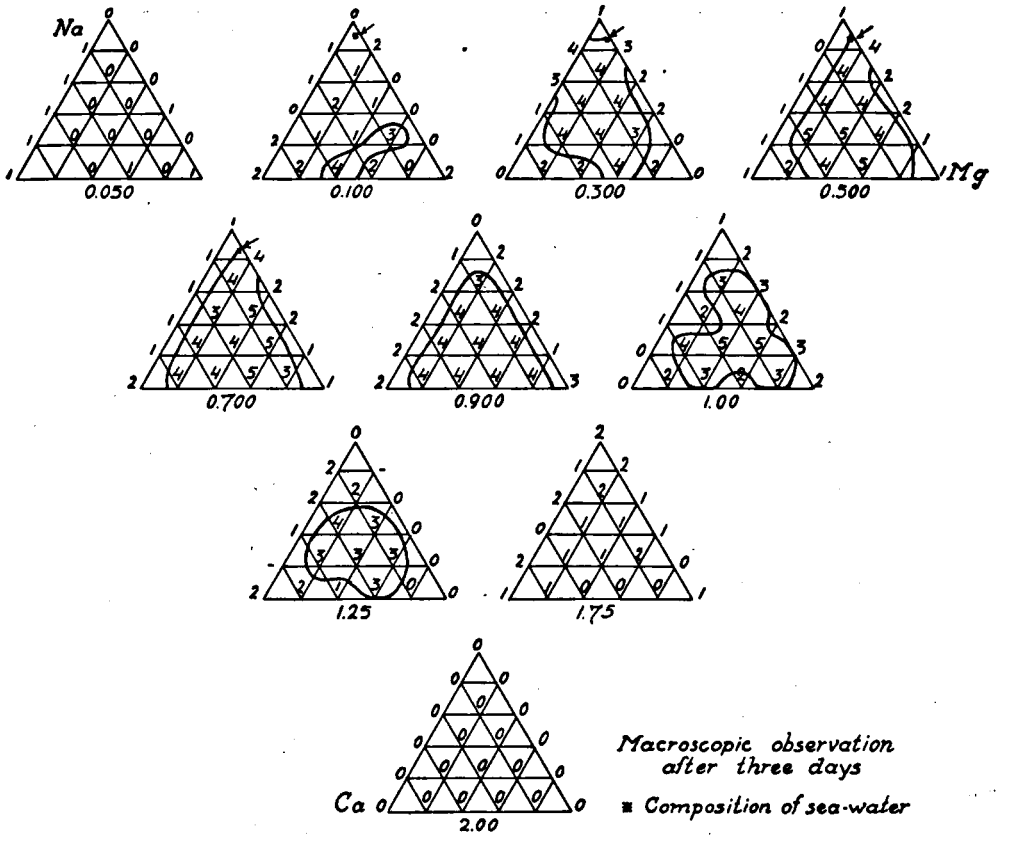

Na
Mg
Ca
0.050
0.100
0.300
0.500
0.700
0.900
1.00
1.25
1.75
2.00
Macroscopic observation after three days
Composition of sea-water

Fig. 2.

after delimiting the regions with figures 3, 4, 5 the following conclusions seem warranted;

1. The concentrations of 0.05, 1.75 and 2.00 eq. destroy the filaments at once. The least toxic concentrations are situated between 0.50 and 0.70 eq. (the concentration of natural seawater being 0.535 eq.).

2. The points 0 (NaCl only), 5 ($CaCl_2$ only) and 10 ($MgCl_2$ only) are noxious to Chaetomorpha, just as the Na-Ca line (1, 2, 3, 4, 5) and almost the entire Na-Mg line (11, 12, 13, 14).

3. The best conditions of the filaments are found in the ternary salt-combinations.

It is remarkable that good conditions are also found on the Mg-Ca line (6, 7, 8, 9). Waters with such a composition, apparently, are not met with in nature after a survey of several thousands of available water-analyses.

Another remarkable fact is that seawater (* in the figure) does not occupy the central place in the "potential milieu" of Chaetomorpha, on the contrary the seawater line approaches the limit of the Chaetomorpha region.

Four days after the inoculation the filaments are observed microscopically. The thickness of the cell-wall was measured (scale 9 μ to one division). These observations are reproduced in table I, in scale-divisions of the ocular micrometer. Each value is the average of at least five measurements. Eventual plasmolysis of the cells is indicated in the same table.

These figures are also plotted in the triangular diagrams (fig. 3). The correspondence of these two series of diagrams is very striking. The comparison of the regions of the different concentrations indicates that antagonism is dependent upon the total salt concentration. This was also concluded by Jacobi and Baas Becking (1933) who studied the incubation time and survival of nauplii of Artemia salina (L.) in combinations of the chlorides of Na, K, Ca and Mg in total concentrations of 2, 2.5 and 3 M. An increasing salt concentration causes a decrease of the potential environment (see also Baas Becking, Karstens und Kanner 1936).

From table I we may conclude that the walls of the cells which are not plasmolyzed and are still in good condition (3) are thin; 0.4—0.6 scale divisions (with some exceptions). As soon as the protoplast has receded from the wall (condition 2, 1, 0) the thickness of the wall increases. This was already mentioned by Kotte (1914) who states that the swelling of the membrane is balanced by turgor-pressure.

TABLE I.

Microscopic observation, four days after inoculation.

Nr.	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition	Wall thickness	Condition
	Series I		Series II		Series III		Series IV		Series V		Series VI		Series VII		Series VIII		Series IX		Series X	
0			1.0	0	1.1	0	1.3	0	1.0	0	1.1	0	1.25	0	1.9	1	1.2	0	1.2	0
1	0.9	0	1.0	0	1.1	2	1.0	0	1.1	0	1.0	1	1.0	0	1.2	1	1.0	0	1.2	0
2	1.0	0	1.0	0	1.0	1	0.95	1	1.0	0	1.0	1	1.2	1	1.1	1	1.1	0	1.1	0
3	0.9	0	1.0	0	0.9	0	1.0	0	1.6	0	1.0	1	1.2	0	1.1	1	1.1	0	1.2	0
4	0.8	0	1.0	0	1.1	0	1.0	0	1.0	0	1.0	1	1.0	0	—	—	1.1	0	1.2	0
5	0.8	0	1.0	0	1.0	0	1.0	0	1.0	0	1.0	0	1.1	0	1.1	1	1.2	0	1.1	0
6			1.0	0	1.0	1	1.0	1	0.5	3	0.6	3	1.0	0	1.3	1	1.1	0	1.2	0
7			1.0	0	0.8	2	1.2	2	0.6	2	1.3	2	1.0	3	1.2	0	1.2	0	1.2	0
8	0.9	0	0.7	0	0.6	2	0.4	3	0.4	3	1.0	3	1.0	2	1.4	2	1.1	0	1.1	0
9			1.0	0	0.8	2	1.0	2	1.2	2	0.8	3	1.0	2	1.2	0	1.2	0	1.1	0
10	1.0	0	1.0	0	0.8	0	1.0	0	1.0	0	1.2	0	1.0	1	1.2	0	1.1	0	1.1	0
11			0.8	0	0.8	0	1.0	0	1.1	1	0.9	0	1.0	1	1.2	1	1.1	0	1.1	0
12	0.8	0	0.9	1	1.0	0	1.0	0	1.0	1	1.0	0	1.0	0	1.2	1	1.2	0	1.2	0
13			1.0	0	1.0	0	1.0	1	1.0	2	1.0	1	1.0	0	1.0	0	1.2	0	1.1	0
14			1.1	1	1.0	1	1.0	0	1.0	2	0.8	1	1.1	0	1.0	0	1.2	1	1.2	0
15			1.0	1	1.0	2	1.0	2	1.0	2	1.0	1	1.0	2	1.0	1	1.2	1	1.1	0
16			1.0	0	0.8	2	0.5	3	0.5	3	1.1	2	1.0	2	0.7	2	1.2	0	1.2	0
17			1.0	1	0.6	3	1.0	2	0.8	3	1.0	2	0.7	2	1.2	1	1.2	1	1.2	0
18			1.0	1	1.0	1	0.5	3	0.8	3	0.8	3	1.0	2	1.4	1	1.1	1	1.0	0
19			0.9	1	1.0	2	0.5	3	0.8	3	0.8	3	1.0	2	1.0	1	1.2	0	1.2	0
20			1.0	1	1.1	2	1.0	1	0.6	3	0.8	3	0.6	2	1.2	1	1.2	1	1.2	0
Average			0.97		0.93		0.92		0.90		0.96		1.05		1.12		1.15		1.15	

Condition; 0: dead.
1: still green, plasmolyzed.
2: beginning plasmolysis, green.
3: no plasmolysis, green.

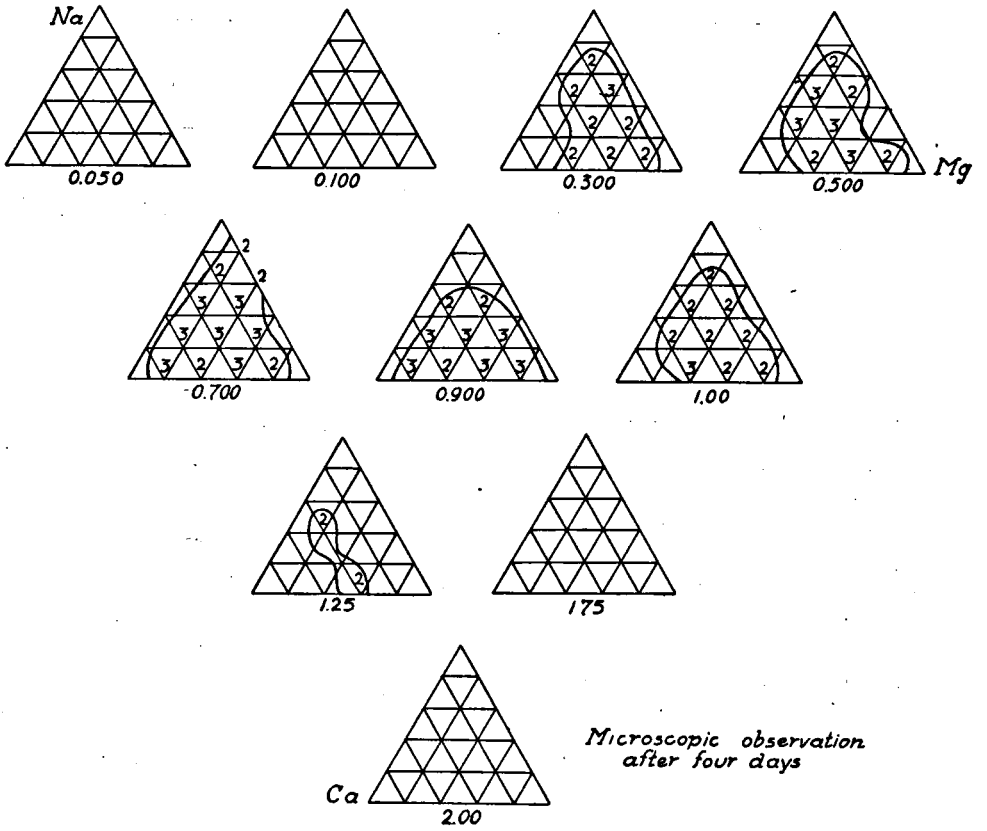

Na
0.050
0.100
0.300
0.500
Mg
0.700
0.900
1.00
1.25
175
Ca
2.00
Microscopic observation
after four days

Fig. 3.

TABLE II.

Nr. of the Series	Condition 0		Condition 1		Condition 2		Condition 3	
	Number	Thickness of the wall	Number	Thickness of the wall	Number	Thickness of the wall	Number	Thickness of the wall
II	14	0.96	7	0.99				
III	8	0.96	4	1.00	8	0.90	1	0.60
IV	9	1.03	4	0.99	4	1.05	4	0.48
V	7	1.10	2	1.05	5	0.96	7	0.63
VI	5	1.04	7	0.97	3	1.13	6	0.80
VII	9	1.07	3	1.07	8	0.91	1	1.00
VIII	5	1.12	13	1.22	2	1.05		
IX	16	1.14	5	1.18				
X	21	1.15						
	94		45		30		19	
Average		1.08		1.08		0.97		0.67

Table II shows the mean values for the thickness of the wall in different conditions. The difference in thickness between the non-plasmolyzed cells (3) and the cells with beginning plasmolysis (2) is apparent.

Table III shows the macroscopic observations after a month, after six and after nine weeks. It appears that the filaments are in a bad condition, while the region in which they are still alive has contracted considerably. After 9 weeks a single filament was found still alive, bright green and nearly without plasmolysis, in concentration series IV ($\pm$ the concentration of natural seawater) at point 16 (NaCl; 12, $CaCl_2$; 12, $MgCl_2$; 6). (See table III next page).

§ 4. *The cell-wall of Chaetomorpha Linum.*

To the data already given in the literature we have to add some remarks about the Chaetomorpha membrane.

Nicolai and Frey-Wyssling (1938) could not obtain a coloration of the filaments with chlorzinc-iodine, neither in fresh material nor in fixed filaments or in those treated with "Eau de Javelle", although the strong birefringence of the cell-wall is an indication for the presence of cellulose, the presence of which substance they were able to show by means of X-ray analysis. However, the wall was intensively coloured by dilute solutions of ruthenium red, indicating the presence of pectin. The pectin present

TABLE III.

Macroscopic observation after a month ! After 6 weeks !! After 9 weeks

Nr.	Series I	Series II	Series III	Series IV	Series V	Series VI	Series VII	Series VIII	Series IX	Series X
0										
1					1					
2										
3										
4										
5										
6			1	1	1!	1				
7				1	1	1	1			
8			1	1	1!					
9				1	2!					
10										
11										
12										
13										
14										
15			1	2!	2!	2	1			
16			1	3!!	2!	2!	1!			
17			1	2	2!	2!	1!	1!		
18			1	2!	3!	1!	2!			
19			1	3!	2!	2!	2!			
20			1	2	3!	1!	2!			

1: still green, plasmolyzed.
2: „ „ beginning plasmolysis.
3: „ „ no plasmolysis.

! indicates the cases in which the filaments after 6 weeks are still green, although they are, nearly all, plasmolyzed. The walls become slimy.
!! the only filament that was still alive after 9 weeks.

was supposed to mask the cellulose, as was also found in the red alga Corallina where Baas Becking and Galliher (1931) could not show the presence of cellulose microchemically; later on Naylor and Russell-Wells (1934) demonstrated macrochemically the precense of cellulose in the walls.

It is interesting that Nicolai and Frey-Wyssling finally obtained positive results with chlorzinc-iodine by leaving the filaments in a concentrated solution of cuprammonia during the night. There was no noticeable decrease of the birefringence and chlorzinc-iodine caused a distinct violet colour after this pretreatment.

We succeeded in isolating the cellulose out of the walls of Chaeto-

morpha after the method of Naylor and Russell-Wells.

"The weed was first heated for half an hour with 1.25 % sulphuric acid under a reflux condenser, then filtered off on a sinter funnel, to avoid possible contamination with cellulose from filter paper, washed free from acid with hot water, and then heated for half an hour with 1.25 % caustic soda. The product was again filtered, washed free from alkali and dried to constant weight in a steam oven".

The two following tests were applied to establish the presence of cellulose;

1. colour test with iodine and sulphuric acid.
2. solubility in cuprammonia, prepared following the receipt of Rosenthaler (1928);

A solution of copper sulphate in water is precipitated with the calculated quantity of sodium carbonate. The precipitate is filtered off, washed repeatedly and dissolved in concentrated ammonia. Afterwards the solution is filtered through a sinter funnel.

After the treatment with 1.25 % sulphuric acid and with 1.25 % caustic soda the filaments gave a bright blue colour with iodine and 66 % sulphuric acid.

In this connection it was pointed out, already by Naylor and Russell-Wells that the iodine solution takes some time to diffuse into the cellulose.

When the filaments obtained after this treatment are observed under the polarization-microscope, two systems are visible. These systems form a nearly rectangular lattice as illustrated in table IV.

TABLE IV.

First system	Second system	Difference
Position of the table	Position of the table	
155°	56°	99°
153°	58.5°	94.5°
129°	41.5°	85.5°
130.5°	42°	88.5°
304°	210°	96°
293.5°	211°	92.5°
292°	206°	84°
204°	120°	84°
298°	209°	89°
297°	205°	92°
301°	212°	89°
		Average 90.4°

Another sample of the Chaetomorpha material was first heated with 2 % caustic soda and afterwards with 5 % caustic soda. After this treatment the filaments gave also a bright blue colour with iodine and sulphuric acid 66 %. The substances that remain on the sinter funnel, after washing free from alkali with hot water, are cellulose and a part of the pectin-like substances (positive reaction with ruthenium-oxychloride). The Schweizers' reagent (cuprammonia) separates the two components by dissolving the cellulose. By evaporating the solution till dry and removing the copper (that was oxidized and black) by a solution of hydrochloric acid and nitric acid and after washing the remaining substance with ammonia, alcohol and caustic soda one obtains the pentosan-containing cellulose.

The cellulose is obtained free from pentosan after the method of König (cited after Rosenthaler 1928);

3 gms. of substance are heated at 133°—135° C. under a reflux condenser with 200 cc glycerol of 1.23 specific weight, to which is added 2 % concentrated sulphuric acid. After an hour heating the product is precipitated with water, boiled up once more, filtered off when still hot and washed with hot water, hot ethanol and a heated mixture of ether and alcohol, until a colourless liquid passes. This cellulose is further purified by means of hydrogen peroxide and ammonia.

To detect the presence of residual pentosan the furfurol reaction was applied. The cellulose was dissolved in 12 % HCl, distilled over and 5 cc of the distillate was used for the furfurol reaction.

In the case of the presence of furfurol a mixture of 2 cc anilin with 5 cc glacial acetic acid and 1 cc of the distillate yields a red colour. As the product obtained with the method of König did not show a red colour, the cellulose was hydrolized, according to the method of Wheldale-Onslow, before the furfurol test was performed.

A small quantity of the isolated cellulose was dissolved in 1 cc of strong sulphuric acid. This solution was poured out into 20 cc distilled water and boiled during an hour, cooled and neutralized with solid $CaCO_3$. Then it was filtered off, the filtrate was distilled over and mixed with a HCl solution so that a 12 % HCl solution was formed. The distillate showed a pale red coloration with two parts anilin and five parts glacial acetic acid, an indicative of the presence of a small quantity of pentose.

From these tests it appears that a great part of the cell-wall consists of cellulose, as was also demonstrated by Nicolai and Frey-Wyssling.

CHAPTER II.

METHODS.

In plasmolyzing the Chaetomorpha filaments in different saccharose-solutions we observed the irregular forms, already described by Höfler (1932) and we also concluded, as Kotte (1914) did, that it was impossible to apply the plasmometric method in this case.

As water-movement, especially in plasmolysis, interested us, two new methods are applied to determine the changes in water-contents.

These methods are;

1. The refractometric method.
2. The dilatometric method.

Next to these two methods there is still a series of direct measurements of the thickness of the cell-wall.

These three methods will be discussed in the following paragraphs.

§ 1. *The refractometric method.*

The starting-point for the so-called refractometric method was the idea that since the phenomena *in* the filament cannot be approached in a quantitative fashion, the changes in the surrounding solution may give an indication of water-exchange. It is evident that the changes in the surrounding solution are so much the greater when the quantity of this solution is relatively small.

When a cell is brought into a hypertonic saccharose-solution, *pure* water is supposed to leave the vacuole until the concentrations of vacuole-sap and surrounding solution are equal. The quantity of water that passes through the cell-wall lowers the concentration of the saccharose-solution. If the quantity of the saccharose-solution is small enough, this dilution may be measured by a difference in refractive index of the plasmolyte, before and after plasmolysis. If the volume of the filament and the quantity of the plasmolyte at the beginning of the experiment are known, the quantity of water excreted into the surrounding solution may be expressed in percents of the volume of the filament.

For the determination of the refractive index the refractometer after Abbe is used. As the refractive index is dependent upon temperature, the refractometer- prisms are kept at a constant temperature by means of a water current, the temperature of which is maintained electrically. After some experience, the instrument allows of a constant reading up to the fourth decimal place.

The following values are found for a solution of 1.36 M. saccharose;

N_D^{20}	1.3938
	1.3939
	1.3938
	1.3938
	1.3937
	1.3938
Average;	1.3938

After provisional tests and several technical changes and improvements, the experiments were carried out in the following way.

A Chaetomorpha-filament is brought into a narrow glass-capillary (about 5 cm long), the capillary being but little wider than the diameter of the filament. The length of the filament is the same as that of the capillary.

At the outset the capillary is filled with the same artificial seawater in which the filaments are cultured in the laboratory. For each experiment a set of ten capillaries was used. To avoid evaporation — this causing a change in concentration and in refractive index of the seawater — the capillaries filled with the filament are placed on glass-rods in a petri-dish filled with a thin layer of seawater.

Then the diameter of each capillary is determined microscopically, at the two ends. For this purpose a microscope was used without front-lens. The condensor is removed and on the microscope table a square cardboard with small holes is placed, through which the capillary is placed in a vertical position. (One division of the scale in the ocular-micrometer; 40 μ).

The average of the values found is noted as the diameter of the capillary.

The diameter of the filament is also determined. The filament is removed from its capillary and mounted in seawater for microscopic observation (scale division $\pm$ 16.5 μ), after which the filament is brought back into its capillary and the capillary is replaced in the petri-dish.

After measurement of the diameters of the ten capillaries and the filaments the plasmolyte is sucked into the capillaries.

As plasmolytes we used saccharose solutions ("saccharose Brocapharm. gegarandeerd zuivere reagentia"). A 1 M. solution was prepared by dissolving 34.2 gr. saccharose to 100 cc seawater. By diluting this stock solution with seawater we obtained the lower concentrations 0.1, 0.2 ..... 0.9 M. For these the intervals in refractive index were determined at the temperature used in the

experiments (15° C., ± 20° C., 26° C.). The higher concentrations were obtained by dilution of a ± 2.5 M. solution of saccharose in seawater, and controlling the concentration refractometrically.

The changing of the solutions in the capillaries was performed in the following way.

A capillary containing seawater and a Chaetomorpha-filament is joined by a piece of thin rubber tubing to a double-pierced stop of a flask filled with tapwater. To the other outlet of this flask suction may be applied, causing the seawater to flow out from the capillary which solution is replaced by saccharose-solution in a similar way. The procedure is repeated several times with saccharose-solution.

The capillaries are now placed in the same order of succession in a petri-dish, the bottom of which is covered with filter paper soaked in the same plasmolyte as applied to the cells.

When the capillaries are filled with a hypertonic solution, the vacuole will contract and water will pass through the cell-wall into the surrounding liquid in the capillaries. (There is no contact between this liquid and the filter paper at the bottom of the petri-dish and the outside of the capillaries is carefully dried, before they are placed in the dish). Accordingly, the refractive index of the ilquid in the capillaries will decrease.

After a definite time, varying with each saccharose-concentration the liquid is blown out of the capillary, by means of a rubber tube, between the prisms of the refractometer and the new refractive index is determined.

If the initial molarity of the saccharose-solution is known, the water-quantity that left the cell may be calculated in volume-percents of the vacuole or of the whole filament per unit of length. For all these experiments, saccharose-solutions are prepared, by dissolving saccharose in artificial seawater. This artificial seawater in which the filaments show almost normal behaviour, we consider as the basal point for the solutions. Thus 1.5 M. saccharose means seawater that contains 1.5 M. saccharose.

The inner diameter of the capillary be 2R.

The diameter of the vacuole be 2r.

The thickness of the cell-wall be a.

The molarity at the beginning of the experiment be m_1.

The molarity at the end of the experiment be m_2.

Then the volume of 1 cm of filament is: $\pi \times 1 \times (r + a)^2$.

The inner volume of the capillary is: $\pi \times 1 \times R^2$ per cm length.

The liquid in this capillary has a volume:

$$\pi \times 1 \times [R^2 - (r + a)^2].$$

The quantity of water passing into the saccharose-solution is put equal to x.

The following formula expresses the relations between the above-mentioned quantities:

$$\pi [R^2 - (r + a)^2] \times m_1 = \{\pi [R^2 - (r + a)^2] + x\} \times m_2.$$

and, therefore

$$x = \frac{\pi [R^2 - (r + a)^2] \times (m_1 - m_2)}{m_2}.$$

When x is reproduced in % of the vacuole-volume (i.e. the volume of the filament within the longitudinal walls) the equation becomes:

$$x = \frac{[R^2 - (r + a)^2] \times (m_1 - m_2) \times 100}{r^2 \times m_2} \%.$$

The final molarity m_2 was calculated in the following way.

As we supposed that the difference in refractive index is caused by an addition of pure water, a dilution-series was prepared for each saccharose-concentration. Within the limits of accuracy of our refractometer, the indices of refraction show a rectilinear relation in regards to dilution.

The decrease in refractive index of the liquid in the capillary by plasmolysis corresponds to a definite percentage of decrease in molarity.

The following example illustrates the procedure.

Refractive index of the saccharose-solution before plasmolysis 1.3859. Refractive index of 60 % of this saccharose-seawater solution + 40 % distilled water;

1.3653
1.3652
1.3653
1.3654
Average; 1.3653

40 % distilled water cause a decrease of 1.3859 — 1.3653 = 0.0206.

1 % dilution of this saccharose-solution corresponds to 515×10^{-6} difference of the refractive index, and the final molarity may be calculated for each capillary in the following way;

TABLE V.

Cap. Nr.	m_1	n_1	n_2	$n_1—n_2$	$\frac{\%\ n_1—n_2}{5.15}$	$m_1—m_2$	m_2
1	1.0	1.3859	1.3828	31	6.02	0.0602	0.9398
2	„	„	1.3845	14	2.72	0.0272	0.9728
3	„	„	1.3831	28	5.44	0.0544	0.9456
4	„	„	1.3836	23	4.47	0.0447	0.9553
5	„	„	1.3833	26	5.05	0.0505	0.9495
6	„	„	1.3837	22	4.27	0.0427	0.9573
7	„	„	1.3826	33	6.41	0.0641	0.9359
8	„	„	1.3835	24	4.66	0.0466	0.9534
9	„	„	1.3828	31	6.02	0.0602	0.9398
10	„	„	1.3836	23	4.47	0.0447	0.9553

A blank series was made to test the reliability of the method. The same technique was applied to the capillaries without filaments. The results of this blank series are mentioned in table VI.

TABLE VI.

	n_1	n_2 Determined after one hour				Average n_2	$n_1—n_2$
		Number of the capillary					
		I	II	III	IV		
A	1.3682	1.3678 1.3679	1.3682 1.3682	1.3683 1.3682	1.3681 1.3681	1.3681	— 1
B	1.3721	1.3720 1.3720	1.3721 1.3721	1.3719 1.3720	— —	1.3720	— 1
C	1.3766	1.3764 1.3764	1.3764 1.3762	1.3763 1.3764	1.3763 1.3762	1.3763	— 3
D	1.3814	1.3814 1.3814	1.3814 1.3815	1.3812 1.3813	1.3815 1.3815	1.3814	— 0

The deviations of the initial refractive index are very small (in the case C the deviation —3 is somewhat larger) and well within the reading error of the refractometer.

At this place the observations of one series will be reproduced in detail, while of the other series the results only will be given [1].

[1] I wish to express my thanks to Miss J. M. Krijthe, M. A. for her assistance in working out this method.

TABLE VII.

Observations of 23-IV-1938. temp.: 15° C.

Cap. Nr.	n_1	n_2	n_1-n_2	$\frac{R}{40}$	r	r + a	time	m_1	m_2	m_1-m_2	% of excreted water
1	1.3859	1.3828	31	8.90	148.17	155.37	30′	1.0	0.9398	0.0602	29.94
2	„	1.3845	14	13.00	139.26	146.46	„	„	0.9728	0.0272	35.89
3	„	1.3831	28	8.70	133.98	139.38	„	„	0.9456	0.0544	32.51
4	„	1.3836	23	8.70	136.95	142.35	„	„	0.9553	0.0447	25.16
5	„	1.3833	26	8.70	144.21	149.61	„	„	0.9495	0.0505	25.25
6	„	1.3837	22	9.10	127.38	134.58	45′	„	0.9573	0.0427	31.44
7	„	1.3826	33	8.10	137.94	145.14	„	„	0.9359	0.0641	30.20
8	„	1.3835	24	8.40	129.36	136.56	„	„	0.9534	0.0466	27.53
9	„	1.3828	31	8.20	145.20	150.60	„	„	0.9398	0.0602	25.80
10	„	1.3836	23	8.90	132.99	140.19	„	„	0.9553	0.0447	28.33

The average percentage excreted into the first five capillaries is 29.75 % (time 30 min.), the average of the percentage excreted into the last five capillaries is 28.66 % (time 45 min.), the average for the ten capillaries is 29.20 %.

With this refractometric method the series of measurements was made, reported in Chapter III.

Plasmolysis was studied in a range of saccharose-solutions at different temperatures.

The time necessary to reach maximal plasmolysis was determined in advance in each case.

§ 2. *The dilatometric method.*

In literature objections are raised repeatedly against volume-determination of cells by observation and measurement of plane-optical sections (Pringsheim (1931), Ernest (1931, 1934), Oppenheimer (1932)).

Hoffmann (1935) gives some graphs, indicating the volume-decrease of several organisms. In his first figure the volume-decrease of Chaetomorpha in a range of low saccharose-solutions is also reproduced. These values were found by measuring the dimensions of optical sections of the cells. It was only possible to calculate the volume in the lower-concentrated saccharose-solutions. In the higher concentrations the form of the cells was too irregular. It seemed necessary, therefore, to find a more exact way to determine the volume of small cells.

For this purpose we used the so-called dilatometer, designed by E. A. Hanson for the voluminometric measurements of small

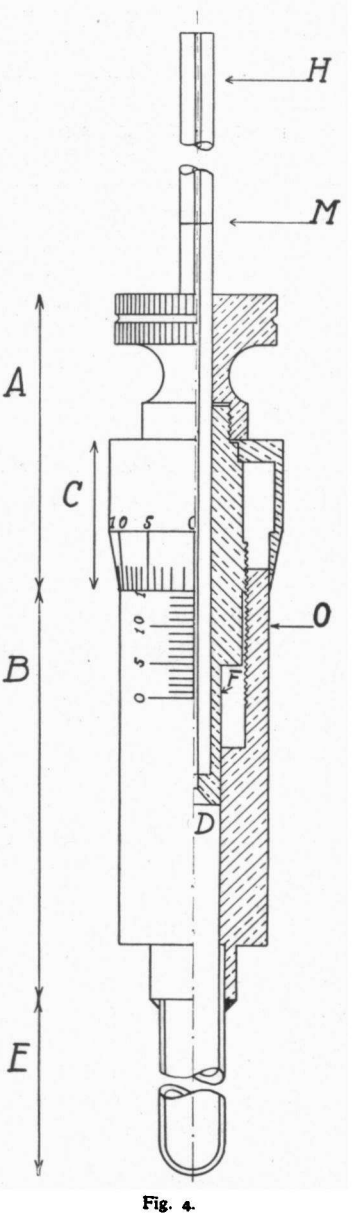


Fig. 4.

Phyllopods performed by Warren, Kuenen and Baas Becking (1938). These authors determined the volume of Artemia salina (L.) by means of a dilatometer in a range of salt concentrations (see fig. 4). In connection with the difference in form between a Chaetomorpha filament and an Artemia our dilatometer was somewhat modified and provided with a glass "appendix". A separate description of this instrument seems, therefore, justified.

The dilatometer was constructed at the Kamerlingh-Onnes Laboratory and modified for our purpose by Mr. A. J. Stuivenberg, Master Mechanic of the Botanical Laboratory.

The apparatus consists of a chromium-plated brass screw-head A, in the axis of which is mounted a glass capillary H by means of Dekhotinsky-cement. A scale is marked upon the overlapping screw-head C. This cap may be screwed upon a metal socket B in which the glass-tube E is sunk. A vernier-scale is engraved upon the rim of the socket.

In order to make a measurement the tube E is filled with solution. The screw-head A is fastened, so that the part F fits into tube D. This part is previously covered with a thin layer of vaseline. The apparatus is immersed into a water bath up to the level O. After temperature-equilibrium has been reached, the head is turned until the meniscus of the water shows at M. The vernier is read and the voluminometer is removed from the bath, unscrewed (care being taken lest any water be lost) and the object to be investigated placed into tube E, after which the apparatus is mounted as described above, and a new reading of the vernier is made.

The filaments before they were brought into the dilatometer, were superficially dried with filter paper in order to remove the adhering water.

It was not so easy to bring the filaments into the tube, without damaging them or touching them by hand.

For this purpose we used a glass-bead ending in a small hook. The filament was folded over the hook and the glass-bead at once sank to the bottom, taking the filament with it. The volume of the glass-bead was determined in advance.

To remove the filament and the glass-bead from the tube after the measurements, a glass-capillary loop is used. During the preliminary tests it was observed that the dilatometer was very temperature-sensitive, the meniscus moved as soon as we touched the tube by hand. To avoid these fluctuations the dilatometer was placed in a thermostat.

A pail was filled up for the greater part and tapwater was streaming slowly into the pail, during the day, whilst the water-volume

was kept constant by a siphon. The dilatometer was kept under water by a perforated board, till mark O and in this way the temperature of the liquid in the tube was kept constant.

The vernier of the dilatometer was calibrated by determination of the volume of a small grain of shot (diameter 3.969 mm).

The dilatometer readings were:

1094.8	—	1022.0	= 72.8	
1082.0	—	1009.5	= 72.5	
1107.1	—	1033.9	= 73.2	
1135.7	—	1063.7	= 72.0	
1135.8	—	1063.7	= 72.1	
1083.0	—	1010.0	= 73.0	Average 72.6.

One division of the vernier scale corresponds, therefore, to 0.4508 mm^3.

§ 3. *Microscopic measurements.*

When, in plasmolysis, the protoplast recedes from the cell-wall the thickness of the wall increases. Water passing through the wall is supposed to imbibe the membrane and to cause this increase. In this case a relation should be expected between the quantity of water, passing through the wall, and the degree of swelling, until a definite maximum is reached. A part of the quantity of water excreted by the vacuole should necessarily remain in the wall, and thus not be found back in the surrounding liquid.

Kotte (1914) mentioned already that the increase in thickness of the cell-wall was caused by a decrease in turgor.

As this problem interested us, swelling was measured in a range of saccharose-solutions after various incubation-periods. (One division of the eye-piece micrometer corresponds to 2.35 μ) [1].

1. In the first place membrane-swelling was investigated in *intact cells.*

For this purpose a filament was observed microscopically in seawater, and the thickness of the membrane was measured in the medium plane of the cell. In the preliminary measurements the cover-glass was paraffined at the four edges to prevent evaporation of the seawater, but as in this way the alga died, this technique was dropped and the cover-glass was supported by glass-needles. After measuring the filament was placed in 1 M. saccharose-

[1] At this place it is a pleasure to thank Mr A. G. L. Adelbert, B.A. for his many faithful and careful measurements.

solution during the night, and next morning the walls were measured again in the plasmolyte.

This procedure was repeated for a whole range of saccharose-solutions. As follows already from the investigations of Kotte the time-factor is not to be neglected in this swelling process. Some observations after 24 and after 48 hours led us to the same conclusion (see table VIII).

TABLE VIII.

M.	Thickness of the cell-wall after 24 hours	48 hours	Difference
1.0	161.2 %	143.7 %	— 17.5 %
0.9	379.7 %	534.2 %	+ 154.5 %
0.8	355.7 %	—	—
0.7	193.4 %	172.6 %	— 20.8 %
0.6	197.5 %	188.2 %	— 9.3 %
0.5	168.9 %	152.2 %	— 16.7 %
0.4	128.7 %	160.0 %	+ 31.3 %
0.3	134.3 %	140.6 %	+ 6.3 %
0.2	117.7 %	127.1 %	+ 9.4 %
0.1	102.1 %	120.1 %	+ 18.0 %

In this way the membrane-swelling was always observed after definite periods.

In the swollen membrane two layers may be distinguished as is already mentioned by Correns, Nicolai and Frey-Wyssling. Swelling (during 24 hours) accentuates the limit between these two layers so that the inner layer appears as a new formation. This layer is too thick, however, to be the product of 24 hours. Only in the case of a strong plasmolysis, when the protoplast has receded from the wall, it may surround itself with a new thin layer.

Noll (1887) already discussed the question of formation of new layers by apposition. For this study he used marine plants. After several futile efforts he tried an impregnation of the cell-wall by means of an insoluble pigment. The pigment was formed as a reaction product, Prussian blue, of two (easily penetrating) soluble substances, potassium ferrocyanide and ferric chloride. Each of these substances was added separately to study their influence upon the material.

40 % $K_4Fe(CN)_6$ did not damage the cells, KCl (the reaction product) proved to be equally harmless in similar concentration. $FeCl_3$, on the contrary, showed a toxic effect, so that an excess

of $K_4Fe(CN)_6$ had to be provided.

The Prussian blue colours the wall very evenly. This points to a soluble modification of the colouring substance. The colour, although soluble, does not diffuse out of the wall, but after an hour it disappeared. This may be caused by the alkaline protoplasm. Colour may return by washing the filament with a solution of $K_4Fe(CN)_6$ in hydrochloric acid.

With this method N o l l found evidence of growth by apposition for the membrane of several species of Caulerpa, Derbesia and Bryopsis.

The same method was applied to the Chaetomorpha membrane. The following solutions were used;

1. $K_4Fe(CN)_6$; 1 part seawater and 1 part 3.2 % $K_4Fe(CN)_6$ in aq. dest.
2. $FeCl_3$ 2 % in aq. dest.

The impregnation was performed in the following way;

1. 10 seconds in $K_4Fe(CN)_6$.
2. rapid washing in seawater.
3. 1 second (at the utmost) in $FeCl_3$.
4. washing.
5. 5 seconds in $K_4Fe(CN)_6$.
6. rapid washing in seawater.
7. 1 second in $FeCl_3$.
8. washing.
9. 3 seconds in $K_4Fe(CN)_6$ (for the excess).
10. washing.

and so on, till the colour shows well.

After an hour the colour of the wall disappeared indeed, but returned after treatment with a mixture of HCl and $K_4Fe(CN)_6$. The filament was brought into an 1 M. solution of saccharose, and after 48 hours the colour returned again after treatment with the hydrochloric-acid solution of $K_4Fe(CN)_6$. After 48 hours no visible apposition of new layers to the cell-wall could be observed, so that the layers, after staying in the plasmolyte for some hours, seem to emanate from the internal side of the membrane. As no apposition, therefore, could be observed, the procedure was discontinued.

K o t t e did not use the intact filaments for his investigation, but the "relaxed membranes", to eliminate the influence of the turgor-pressure. These "relaxed membranes" he obtained as follows; the filaments were cut in parts of 2 cm long, on a glass-plate and in each of those parts 7—10 cells were deflated by a razor cut. The edge of the razor was groined in that way that the ends touched the glass-plate, but the part between the two ends was removed about

0.2 mm from the plate. By doing this a total severing of the algal filament (diameter about 0.3 mm) into parts was prevented.

K o t t e observed for the Chaetomorpha-membrane a decrease in swelling corresponding to an increase of the saccharose-, NaCl- and seawater-concentration.

From his data we conclude a decreasing hydrating action of the higher concentrations.

In the case of membrane-swelling in intact cells, several factors act in the same or in opposite direction;

a. in lower concentrations (no receding of the protoplast)
   1. decrease of turgor pressure causes increase of membrane-thickness.
   2. quantity of water passing through the wall causes increasing membrane-thickness.
   3. increasing concentration of the plasmolyte (i.e. decreasing hydration of the wall-substances) causes decrease of membrane-swelling.

b. in higher concentrations (the protoplast has receded from the wall, turgor-pressure plays no longer a rôle) the factors 2 and 3 are acting in opposite direction.

Thus the graph reproducing the swelling is the resultant of several relations. Turgor-pressure and decreasing hydration (of the wall substance by the high-concentrated plasmolyte) counteract the swelling, whereas it is advanced by the quantity of water passing through the wall excreted by the shrinking vacuole.

In this connection it would be worth-while to determine, if possible, the influence of these three factors separately.

That part of the quantity of water excreted by the vacuole that passes into the surrounding liquid is found by the refractometric method.

The influence of the hydration may be found by observing the swelling of the wall in pricked (deflated or relaxed) cells.

2. Measurements of the swelling in *relaxed membranes.*

The method of pricking was as follows;

First the thickness of the wall in 10 living cells was measured and its average determined. Then each cell is pricked under low magnification with a preparation-needle, provided with a very thin and keen, curved point. With the aid of a metal loop the filament was kept in place, and the pricks were made in the centre of the loop.

After this the filaments were brought into a saccharose-solution in which they remained for 24 hours. In the higher concentrations

the layers were damaged to such an extent that no exact measurements were possible. The lamellae were separated and between them spaces appeared.

A second series of observations was performed after the filaments had remained in the saccharose-solution during one hour. In each filament a number of cells was left intact for comparison of the swelling of the membrane in living and in pricked cells.

A disadvantage of this method is that the observation of filaments in a range of saccharose-solutions from 0.1—1.0 M. (with intervals of 0.1 M.) takes at least three days. During this period the temperature in our laboratory varied considerably. Therefore we preferred to repeat these observations in a room of constant temperature. At the same time we obtained the influence of temperature on the swelling.

In the series mentioned above the effect of saccharose-seawater solutions on membranes of intact and of pricked cells was studied. In the following series the influence of turgor-pressure will be investigated. Up till now no notice was taken of the changes in the thickness of the wall at the moment after the pricking. When, as K o t t e assumed, the swelling of the membrane is counteracted by the turgor-pressure, it is obvious that the release of this pressure expresses itself in a sudden thickening of the membrane. The hydrating action of the saccharose-solution is supposed to be a time reaction, so that this influence at the moment of the pricking will be almost zero.

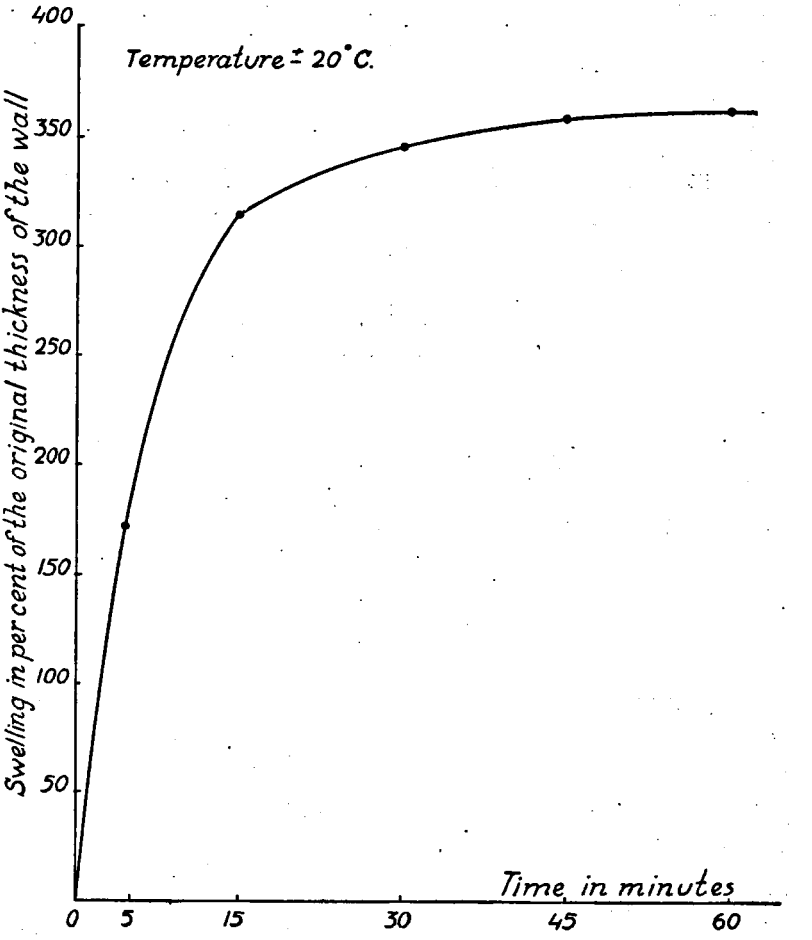
In this way the influence of turgor-pressure and the hydrating action of the plasmolyte on the membrane swelling will be investigated separately. We find the impeding influence of the turgor-pressure by measuring swelling directly after pricking.

Table IX (graph 1) demonstrates a strong swelling directly after the pricking, but the maximum is only reached after about an hour, when the swelling action of the seawater is completed.

TABLE IX.

Time after the pricking	Swelling in seawater expressed in % of the cell-wall in the intact cell
5 min.	173.15
15 „	316.67
30 „	344.44
45 „	358.33
60 „	367.60

In the same way turgor-pressure in the range of plasmolytes, that do not cause visible plasmolysis, was investigated. The filaments



Graph 1.

were left in the plasmolytes for about an hour, than pricked and the increase in thickness determined immediately after the pricking.

The order of the successive manipulations for these measurements was;

1. Measurement of 20 consecutive cells in a filament (40 walls).
2. Pricking of the cells 1—10.
3. Filaments in plasmolyte during an hour.
4. After this hour measurements of the cells 1—20.
5. Of the intact cells (11—20) the number of plasmolyzed cells is determined.
6. Pricking of the intact cells 11—20.
7. Measurements of the pricked cells 11—20 directly after the pricking.

4. gives the total thickness of the wall in intact cells after an hour, and the total thickness of the wall in the pricked cells. This latter value, diminished by 100 % indicates the swelling in consequence of the hydrating action of the plasmolyte and the abolishing of the turgor-pressure.

When this value is diminished once again by the increase in thickness, when turgor-pressure is abolished, we find the influence of the plasmolyte.

The value for the impeding action of the turgor-pressure is found by diminishing the values for the wall-thickness at point sub 7 by the values of the intact cells 11—20 at point sub 4.

These measurements were repeated at different temperatures.

3. Finally a series of microscopic measurements of the length and the width of the intact cell was carried out. It was our intention to measure changes in length in the range of saccharose-solutions kymographically. But this proved to be impossible as will be described in § 4. For that reason we only had recourse to microscopic measurements. In the higher concentrations, however, it was totally impossible to measure exactly volume-changes, as the form of the plasmolyzed cells was far too irregular. In the lower concentrations the forms are still regular, but the differences in length and width are so small that most of them do not surpass the observational error (1.14 %). The results of these measurements are only mentioned with due reserve.

§ 4. *Measurements of length with the kymographion.*

It interested us to know if the length of a Chaetomorpha filament changed in the several plasmolytes and whether a relation existed between the concentration of the plasmolyte and the change in length of the filament.

The Chaetomorpha-filament is stretched between two small rings. The one attached at the bottom of the vessel in which the Chaetomorpha filament is surrounded by the plasmolyte, at the other ring round the filament just above the surface of the

plasmolyte a cotton thread is attached. The other end of this thread is fixed to one arm of a lever, the second arm ends in a recording needle, suitable magnification being obtained in the usual way. The needle is balanced by some small weights, so that the filament is just stretched but not drawn out. However, it proved nearly impossible to reach this condition, as the filaments were not totally straight but rather tortuous. In fact, small differences in length were observed, but we do not suppose these values to be at all significant, the method being far too coarse to register minute longitudinal changes of a small part of a filament.

CHAPTER III.

MEASUREMENTS IN SACCHAROSE-SEAWATER SOLUTIONS.

In this chapter a report will be given of the measurements obtained with the methods, described in Chapter II.

§ 1. *Measurements by means of the refractometric method.*

In the first place the quantity of water, excreted by filaments in 1.17 M. saccharose-seawater during 30 minutes was determined (table X) for material kept in culture in the laboratory

I during 4 months
II during 2 months
and III recently received from Den Helder.

TABLE X.

Percentage of water excreted by Chaetomorpha filaments in 1.17 M. saccharose-seawater.

I	II	III
19.89	31.24	31.77
26.26	31.66	32.42
40.60	35.24	30.87
28.58	34.46	31.63
39.01	25.92	33.96
33.71	30.10	34.48
45.45	30.12	42.84
29.77	38.31	35.62
Average; 32.91 %	32.13 %	34.20 %
3σ; 24.96 %	10.65 %	10.77 %

Before this experiment a layer of blue algae which totally covered the 4 month old material was removed. We see the correspondence between the groups I and II, while the percentage, excreted in group III (the fresh material) is somewhat greater.

Table XII and graph 2 reproduce the values at room-temperature ± 20° C. In the first place the nearly rectilinear course between 0.8 M. and 2.2 M. saccharose-seawater is striking. At 0.8 M. a beginning receding of the protoplast from the wall is visible in all filaments after half an hour (see table XI).

TABLE XI.

10-3-1938	% Plasmolyzed cells after different periods										
M.	5'	10'	15'	20'	25'	30'	35'	40'	45'	50'	55'
1.0	97	100									
0.9	86	100									
0.8	77	93	93	100							
0.7	23	43	45	59	66	66	66		75		100
0.6	3	3	3	3	3		3			3	
0.5											
0.4											
0.3											
0.2											
0.1											

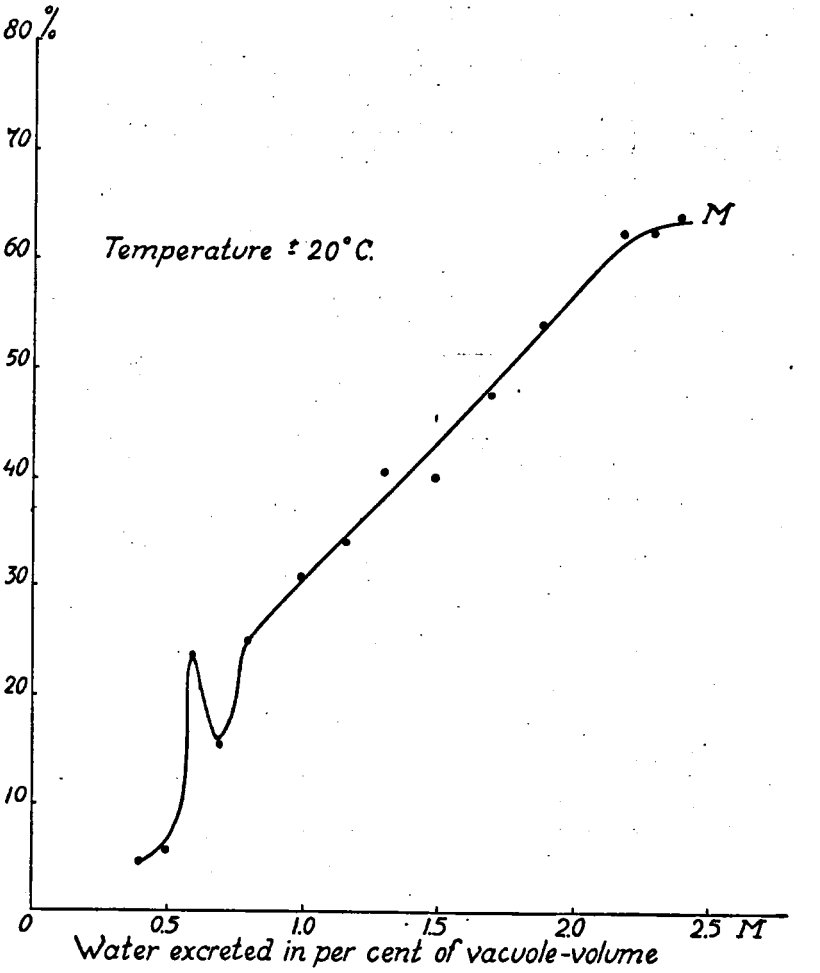
This value of the visible plasmolysis is, however, not constant, in July and August the recession of the protoplast was already etablished in 0.6 M. saccharose-seawater solutions, and perhaps it will also vary for younger and for older cells.

In the concentrations lower than 0.8 M. we find a discontinuity in the curve, with a top at 0.6 M. As will be shown in the following pages, this discontinuity is found also at other temperatures. The value, found for 0.2 M. is to be discarded, as for this low concentration the observational error in the reading of the refractometer is considerably high.

As we supposed that temperature influenced the results obtained with the refractometric method, some series were made at constant temperature.

Table XIII and graph 3 give some values, found in the laboratory at constant temperature (26° C.). (See table XIII, page 310).

In graph 3 the rectilinear relation between concentration and percentage of water-excretion in the region 1.0 M.—2.3 M. appears



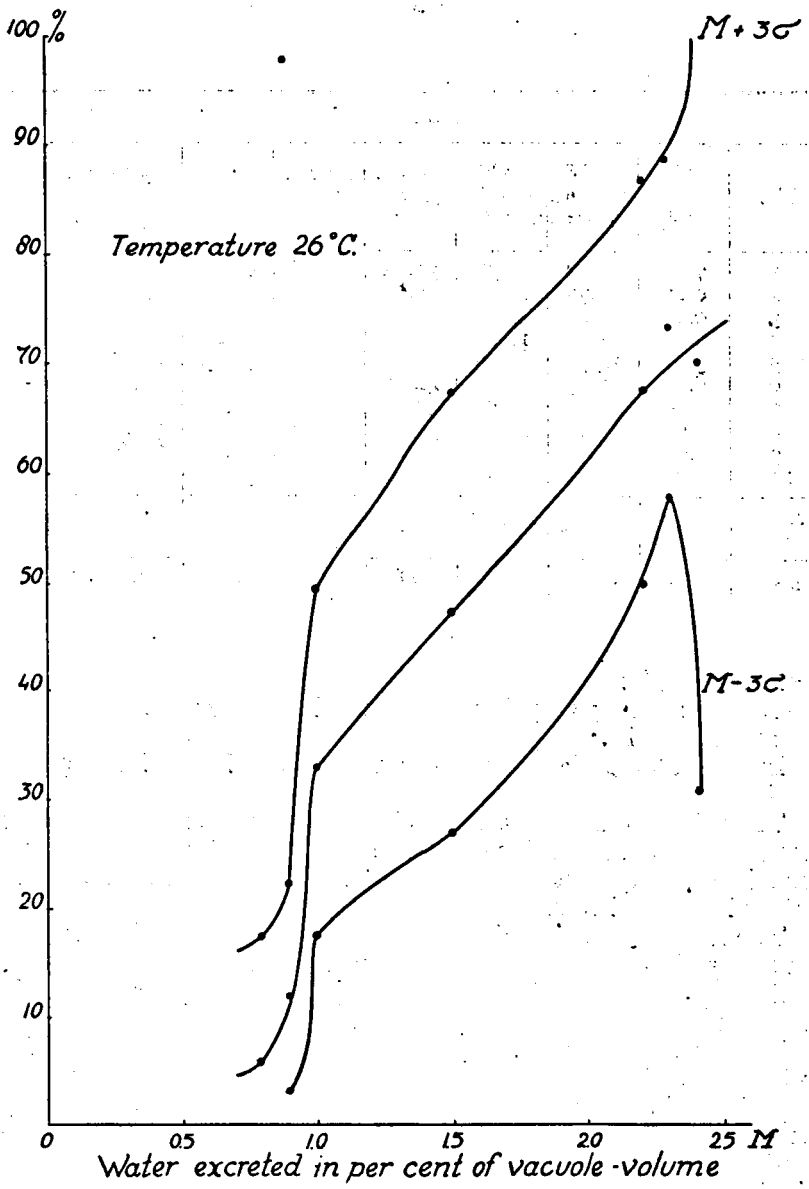
Graph 2.

again. In the concentrations below 1.0 M. the graph shows a considerable discontinuity. The values at this high temperature are somewhat higher than at room-temperature:

TABLE XII

Percentage of water-excretion in a range of saccharose-seawater solutions at room-temperature ± 20° C.

Nr.	0.2 M. 2 hours 2-8-'38	0.4 M. 2 hours 1-8-'38	0.5 M. 1 hour 31-7-'38	0.6 M. 1 hour 30-7-'38	0.7 M. 45 min. 29-7-'38	0.8 M. 45 min. 28-7-'38	1.03 M. 45 min. 14-6-'38	1.17 M. 30 min. 25-8-'37	1.30 M. 30 min. 16-7-'37	1.5 M. 20 min. 8-3-'38	1.7 M. 20 min. 26-6-'37	1.9 M. 20 min. 16-2-'38	2.2 M. 15 min. 1-3-'38	2.3 M. 10 min. 7-3-'38	2.4 M. 15 min. 21-2-'38
1	3.73	3.24	2.25	34.78	16.50	34.98	40.81	31.77	42.40	38.65	44.21	53.45	55.94	68.36	54.21
2	0.00	—	1.89	23.93	15.96	24.12	30.85	32.42	42.42	36.75	45.15	63.87	66.01	69.15	67.12
3	16.23	4.01	3.36	13.16	14.40	22.21	31.52	30.87	36.30	52.06	34.17	52.76	55.64	66.66	78.09
4	8.42	1.79	4.94	17.89	17.17	26.24	27.03	31.63	44.94	33.58	47.12	51.65	77.95	60.74	71.59
5	6.00	2.85	6.89	26.66	14.14	24.37	32.92	33.96	38.51	31.16	57.92	59.50	67.34	60.29	64.64
6	9.24	9.63	2.29	31.06	10.15	23.81	36.98	34.48	41.00	33.59	57.83	51.53	57.21	50.65	62.75
7	—	5.71	7.79	21.70	14.09	13.88	29.20	42.84	39.26	47.48	49.01	55.54	61.29	57.19	64.89
8	3.32	6.15	10.79	22.65	15.13	24.33	24.50	35.62	43.44	36.39	47.44	50.60	61.13	68.15	61.12
9	6.76	5.85	5.39	24.91	17.32	21.49	27.11	—	39.84	55.08	50.30	51.85	61.93	62.36	56.10
10	3.98	—	6.78	22.02	18.01	29.25	28.90	—	38.38	33.55	43.37	51.94	—	62.25	58.36
Average	(6.41%)	4.65%	5.24%	23.88%	15.29%	24.47%	30.98%	34.20%	40.65%	39.83%	47.65%	54.27%	62.72%	62.58%	63.89%


100 %
90
80
70
60
50
40
30
20
10
0
0.5
1.0
1.5
2.0
2.5 M
M + 3σ
M - 3σ
Temperature 26°C.
Water excreted in per cent of vacuole-volume

Graph 3.

TABLE XIII.

Nr.	Percentage of water-excretion at 26° C.							
	0.7 M. 45 min. 26-3-'38	0.8 M. 30 min. 23-3-'38	0.9 M. 15 min. 25-3-'38	1.0 M. 30 min. 14-3-'38	1.5 M. 20 min. 15-3-'38	2.2 M. 15 min. 16-3-'38	2.3 M. 10 min. 22-3-'38	2.4 M. 6 min. 21-3-'38
1	7.05	7.04	7.20	31.44	46.96	69.74	67.17	56.62
2	0.00	5.73	13.33	37.69	44.45	66.87	71.86	76.10
3	0.00	8.09	10.30	31.40	42.82	62.63	—	85.74
4	8.64	—	11.82	22.29	54.59	78.46	75.17	91.61
5	5.02	14.97	11.43	29.25	61.82	76.63	66.87	66.91
6	6.51	1.65	8.95	42.58	58.43	—	79.26	—
7	—	5.94	15.57	35.53	39.40	58.08	71.49	69.00
8	0.00	1.02	16.23	31.23	37.00	65.73	70.23	45.40
9	8.92	2.52	16.92	34.93	40.83	64.73	82.67	70.79
10	5.33	4.23	—	35.46	44.72	71.72	76.97	68.20
Average	4.61%	5.69%	12.62%	33.18%	47.10%	68.28%	73.52%	70.04%
3σ	11.73%	12.03%	9.51%	15.48%	19.92%	18.60%	15.27%	39.39%

For example:
$\pm$ 20° C. 2.2 M.; 62.7% of vacuole-volume, excreted
26° C. 2.2 M.; 68.3% „ „ „

In graph 3, besides the mean values, the limits $M \pm 3\sigma$ are indicated.

The third and fourth series of observations were made in a cellar room on the North-side of the laboratory, where temperature remained nearly constant at 15° C. The results are reproduced in tables XIV and XV and in the graphs 4 and 5.

The first series at 15° C. was carried out with material that had been kept in culture already for some weeks in the laboratory.

In graph 4 the rectilinear relation (1.2—2.2 M.) appears again and also the discontinuity at 0.8, 0.9 M.

The material of the second series, graph 5, was fresh and for this whole series the material of the same shipment was used, within a period of three weeks so that these values seem satisfactorily comparable. Between 1.0 and 2.2 M. the relation between concentration and water-excretion is again rectilinear and again a discontinuity at 0.7—0.9 M. is found.

In the bottom row of table XV the water-excretion is expressed in percents of the volume of the whole filament (graph 5 +). It

TABLE XIV.

Percentage of water-excretion at 15° C. — 1st Series.

Nr.	0.7 M. 45 min. 30-3-'38	0.8 M. 45 min. 2-4-'38	0.9 M. 30 min. 3-4-'38	1.0 M. 30 min. 4-4-'38	1.2 M. 25 min. 6-4-'38	1.5 M. 15 min. 8-4-'38	2.0 M. 15 min. 11-4-'38	2.3 M. 10 min. 12-4-'38	2.6 M. 8 min. 13-4-'38
1	—	6.19	4.13	12.06	23.25	27.70	45.66	58.03	47.70
2	32.07	8.08	1.99	6.99	21.50	24.15	47.80	51.66	56.51
3	8.00	15.12	3.22	13.07	21.48	27.04	47.60	49.93	49.46
4	11.63	6.96	5.44	11.43	23.15	—	55.81	52.42	55.76
5	12.94	8.71	7.54	15.19	22.23	24.53	50.79	46.07	62.86
6	22.57	6.16	9.56	12.71	26.82	30.10	54.53	55.61	41.13
7	10.30	11.36	5.68	13.18	34.70	—	41.87	48.80	49.49
8	14.66	8.59	8.29	—	24.08	30.65	38.02	—	55.07
9	16.04	9.12	10.57	16.60	21.18	31.60	42.29	43.79	—
10	19.27	5.75	3.67	10.55	21.57	46.96	42.42	53.66	62.08
Average	16.39 %	8.60 %	6.01 %	12.42 %	24.00 %	30.34 %	46.68 %	51.11 %	53.34 %

TABLE XV.

Percentage of water-excretion at 15° C. 2nd Series.

Nr.	0.2 M. 3 hours 2-5-'38	0.4 M. 2 hours 30-4-'38	0.5 M. 60 min. 28-4-'38	0.6 M. 60 min. 27-4-'38	0.7 M. 45 min. 22-4-'38	0.8 M. 45 min. 26-4-'38	0.9 M. 45 min. 25-4-'38	1.0 M. 30 min. 23-4-'38	1.3 M. 20 min. 7-5-'38	1.5 M. 15 min. 21-4-'38	1.8 M. 12 min. 6-5-'38	2.0 M. 15 min. 29-4-'38	2.5 M. 12 min. 3-5-'38
1	4.91	0.00	6.41	16.58	24.03	18.78	25.54	29.94	38.62	39,64	53.00	48.44	67.54
2	0.00	0.00	5.28	8.77	26.99	10.53	15.05	35.89	49.72	39.06	37.44	65.95	66.01
3	0.00	0.00	4.95	7.53	22.05	16.62	18.73	32.51	36.22	41.30	54.15	61.29	62.06
4	11.10	2.45	6.66	4.60	16.86	22.76	—	25.16	33.26	49.98	52.00	53.47	58.70
5	0.00	0.00	6.52	9.49	20.03	17.38	15.60	25.25	34.47	46.25	—	47.32	66.78
6	0.00	0.00	6.49	13.72	22.93	17.62	13.90	31.44	36.76	31.40	43.84	56.06	53.66
7	0.00	6.40	11.86	—	20.23	15.13	21.82	30.20	38.16	42.11	46.10	55.33	60.21
8	3.13	11.98	15.12	6.18	14.78	19.28	17.84	27.53	36.08	50.59	48.96	57.62	62.98
9	0.00	0.00	7.40	7.48	15.65	14.72	20.13	25.80	31.76	34.02	51.28	55.13	77.92
10	1.46	5.92	7.86	9.44	12.66	19.31	14.14	28.33	33.72	45.08	44.37	54.48	—
Average	2.06%	2.67%	7.85%	9.31%	19.62%	17.21%	18.08%	29.20%	36.88%	41.94%	47.90%	55.51%	63.98%
3σ	—	—	9.06%	10.53%	13.23%	9.36%	11.02%	9.93%	14.13%	17.88%	15.36%	15.57%	22.65%
V.	1.36%	2.29%	7.12%	8.81%	18.51%	16.14%	16.03%	27.22%	33.48%	37.74%	45.78%	51.62%	58.45%

V indicates the water-excretion in the capillaries, in percentage of the volume of the whole filament.

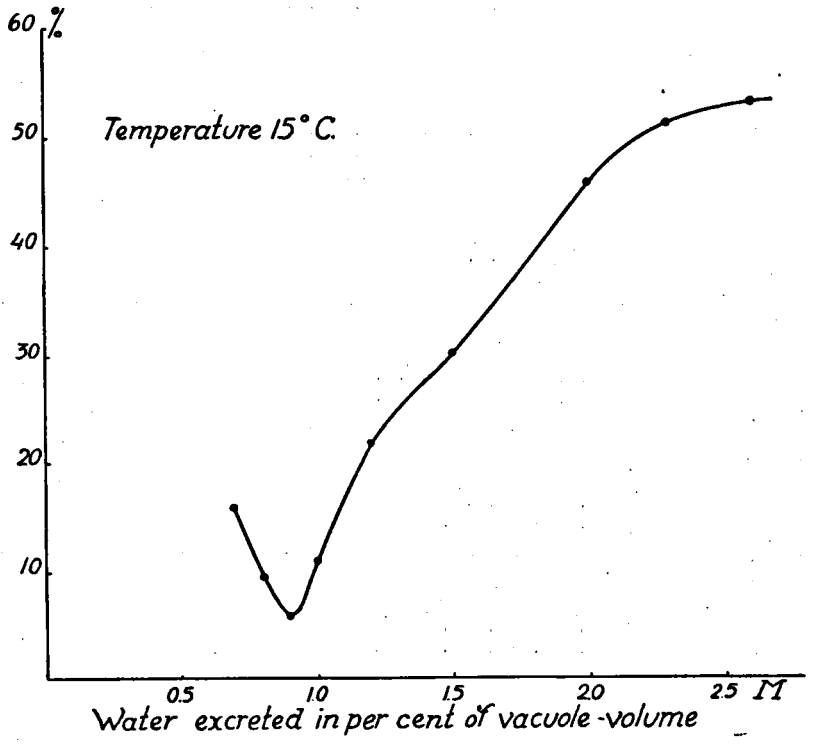
TABLE XVI.

Volume change in % of the original volume in saccharose-seawater at ± 20° C.

Nr.	0.15 M. 2½ hour 15-7-'38	0.35 M. 2½ hour 21-6-'38	0.5 M. 1 hour 28-9-'38	0.6 M. 1 hour 29-9-'38	0.7 M. 45 min. 30-9-'38	0.8 M. 30 min. 1-10-'38	0.9 M. 30 min. 2-10-'38	1.0 M. 30 min. 2-7-'38	1.2 M. 20 min. 4-7-'38	1.5 M. 10 min. 16-7-'38
1	— 1.9	+ 3	— 2.4	— 2.0	— 2.7	— 1.9	— 11.1	— 18.2	— 40.6	— 34.5
2	+ 0.2	+ 1.5	— 2.1	0.0	— 8.8	— 3.2	— 8.9	— 30.4	— 40.1	— 37.0
3	— 0.5	+ 2.4	+ 1.5	— 4.0	+ 1.1	— 2.0	— 5.9	— 22.7	— 41.8	— 28.6
4	— 0.9	+ 3.9	+ 1.0	+ 3.5	— 4.9	+ 0.8	— 6.3	— 34.6	— 37.4	— 50.7
5	+ 2.5	+ 2.9	— 2.4	— 3.5	— 6.1	+ 5.6	— 9.0	— 24.0	— 34.6	— 34.0
6	—	+ 2.2	+ 0.6	+ 0.4	— 5.0	+ 2.3	— 9.4	— 31.1	— 35.7	— 27.4
7	— 0.2	+ 4.2		— 0.5	— 2.2	+ 0.7	— 14.9	— 25.7	— 40.0	— 31.9
8	— 3.6	+ 3.5		+ 1.6		— 3.5		— 28.0	— 38.4	
9	+ 2.2	+ 3.7		+ 6.0		— 1.8		— 29.8	— 39.0	
10	+ 0.5	+ 3.2		— 5.4		— 4.1		— 32.5	— 41.5	
11		+ 2.4						— 36.1	— 40.6	
12		+ 0.5						— 42.1	— 39.4	
13		+ 0.8						— 28.0	— 37.0	
14								— 34.0	— 36.7	
15								— 20.8	— 29.0	
16								— 28.1	— 32.5	
17								— 21.9	— 46.1	
18								— 32.7	— 42.9	
19								— 27.7	— 44.5	
20								— 32.4	— 36.8	
Average	— 0.1 %	+ 2.5 %	— 0.6 %	— 0.4 %	— 4.1 %	— 0.7 %	— 7.9 %	— 29.0 %	— 37.7 %	— 34.9 %
3σ	5.37%	3.33%	5.07%	10.38%	8.79%	8.64%	15.15%	16.92%	12.15%	21.51 %

does not change the form of the curve, all values are somewhat lower, however.

Graph 6 shows the four curves obtained by means of the refractometric method. A comparison of the two series at 15° C. shows



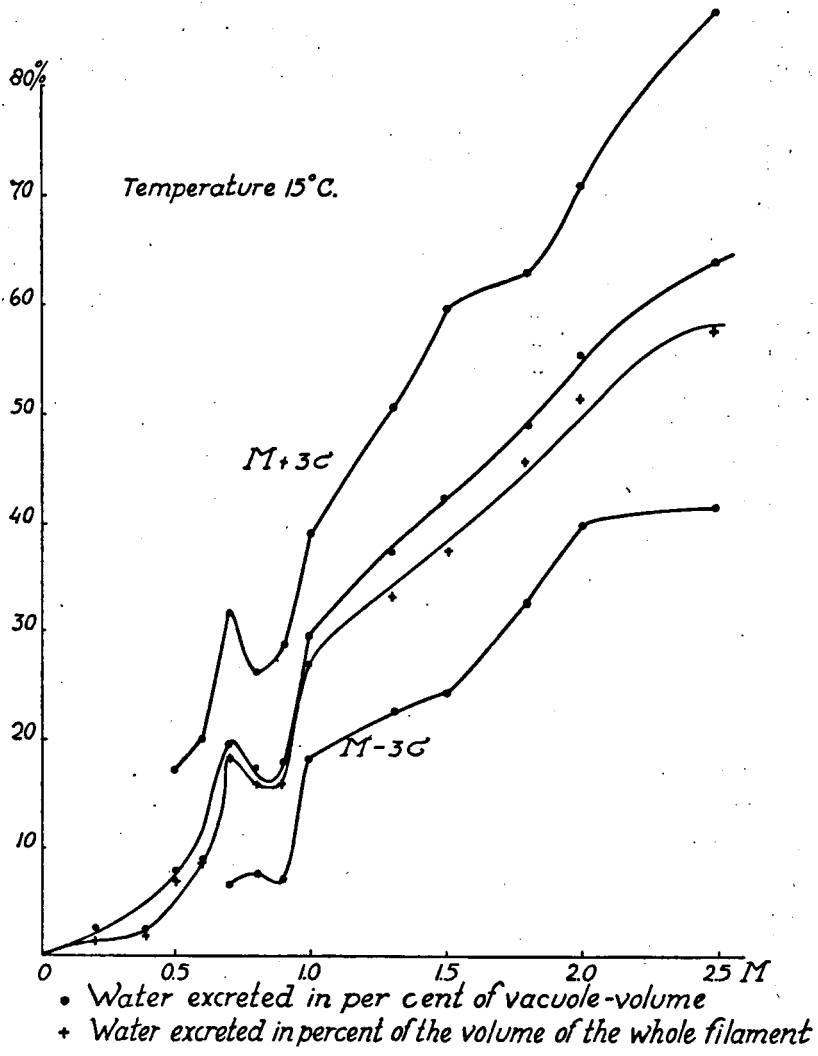
Graph 4.

that the values of the fresh material are somewhat higher but the form of the two curves is the same.

In comparing curve IV (± 20° C.) and curve I (15° C.) we see the correspondence of the curves in the regions between 1.0 and 2.4 M., whereas the values in curve III (26° C.) are higher.

§ 2. *Measurements by means of the dilatometric method.*

Table XVI and graph 7 show the change in volume of Chaetomorpha filaments in a range of saccharose-seawater solutions.

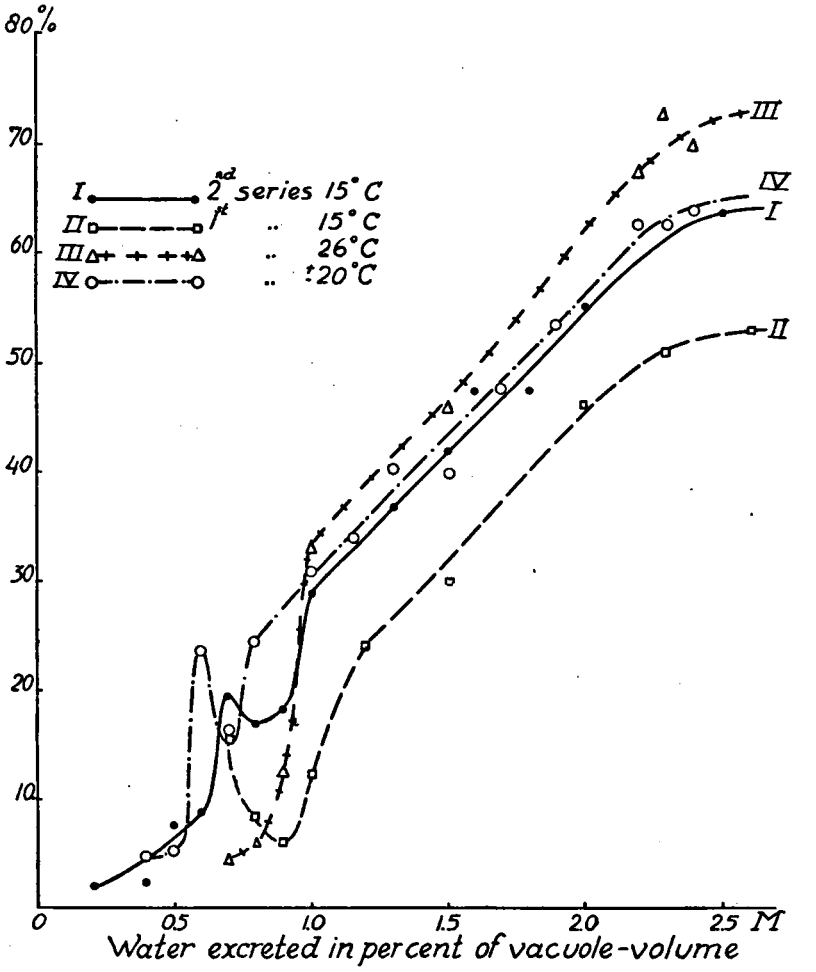


Graph 5.

As is shown in graph 7 the dilatometric method yields the same discontinuity between 0.7 and 0.9 M.

§ 3. *Microscopic measurements.*

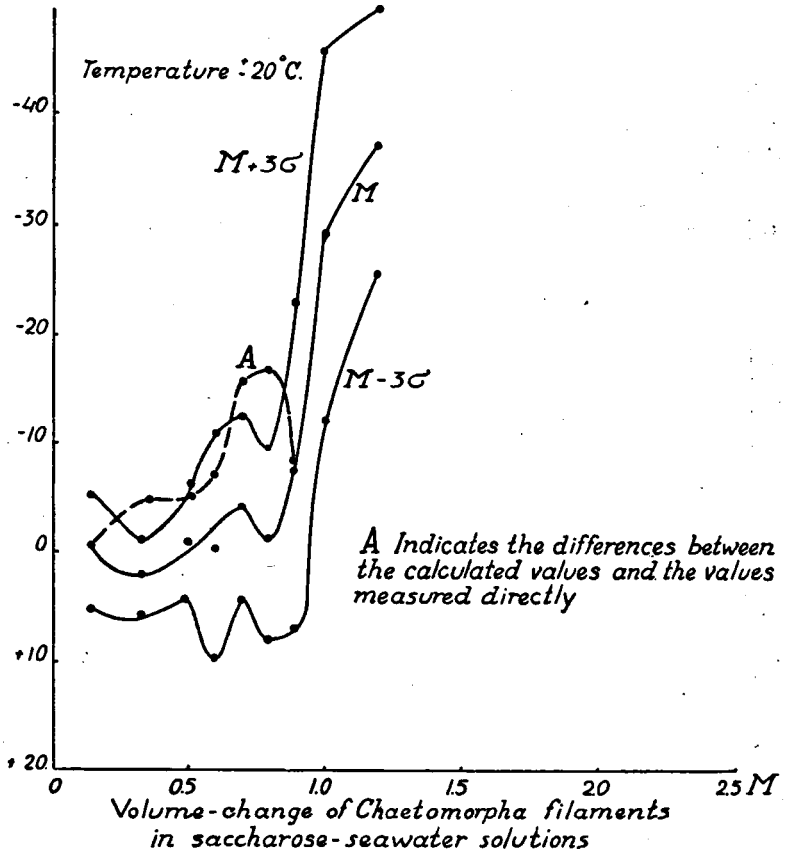
1. In the first place the membrane-swelling in *intact cells* in a range of saccharose-seawater solutions was measured after different



Graph 6.

periods of sojourn at room temperature, see table XVII, graph 8. Each value is the mean of at least 20 measurements of cell-walls. (See table XVII page 319).

In the lower concentrations the curves rise rather slowly, but from 0.5 M. onward they rise steeply and all show a maximum at 0.8 M. From this point they descend in all cases.

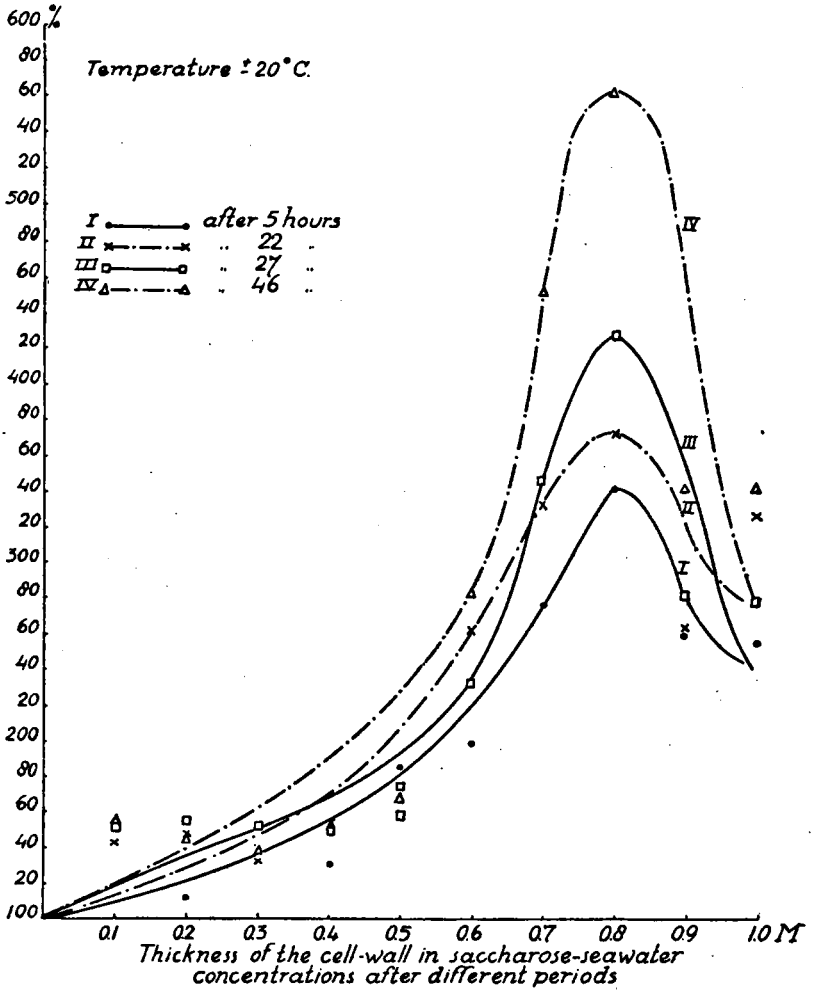


Volume-change of Chaetomorpha filaments in saccharose-seawater solutions

Graph 7.

2. The measurements were repeated for *relaxed membranes*. For this purpose the thickness of the wall of a number of cells was determined and after the cells were pricked as described in Chapter II, they were brought into the saccharose-seawater solutions and after different periods their wall thickness was again determined (see table XVIII, graph 9).

These high swelling values may be explained by the excessive hydration of the lamellae, after that long period in the plasmolyte.



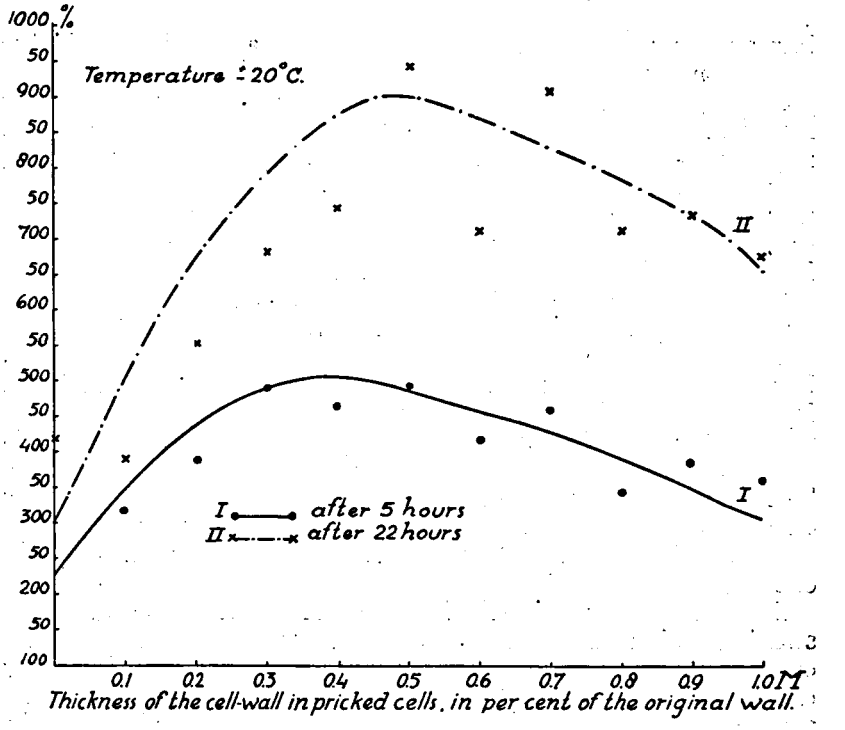
Graph 8.

The lamellae are separated and it is very difficult, therefore, to measure the thickness exactly, especially as it varies considerably between the transversal wall and the middle of the cell. The

TABLE XVII.

Thickness of the wall in a range of concentrations at ± 20° C. in per-cent of the original thickness.

M. saccharose	After 5 hours	After 22 hours	After 27 hours	After 46 hours
	I	II	III	IV
0.1	112	143	150	155
0.2	112	148	156	143
0.3	145	132	152	128
0.4	130	154	150	151
0.5	184	158	174	167
0.6	197	262	232	282
0.7	272	333	347	453
0.8	342	371	427	564
0.9	258	261	282	341
1.0	254	327	279	342



Graph 9.

TABLE XVIII.

Thickness of the wall in pricked cells, in percent of the original wall-thickness. Temperature ± 20° C.

M. saccharose	After 5 hours	After 22 hours
0.0	296	414
0.1	316	391
0.2	420	552
0.3	480	681
0.4	461	741
0.5	482	940
0.6	414	708
0.7	456	908
0.8	343	710
0.9	387	730
1.0	359	675

lower concentrations have a higher hydrating action than the higher concentrations.

After 5 hours the swelling action of the plasmolyte has not yet reached its maximum. The curves show a (not very pronounced) maximum at about 0.4—0.5 M. saccharose. As, already after 4 hours, especially in the lower concentrations, an exact measurement, was nearly impossible, the measurements were repeated for a series with a sojourn of an hour in the plasmolyte. In this case 10 cells in each filament were pricked and for 10 others the swelling in intact cells was measured, so that the two curves in graph 10 are directly comparable (see also table XIX.)

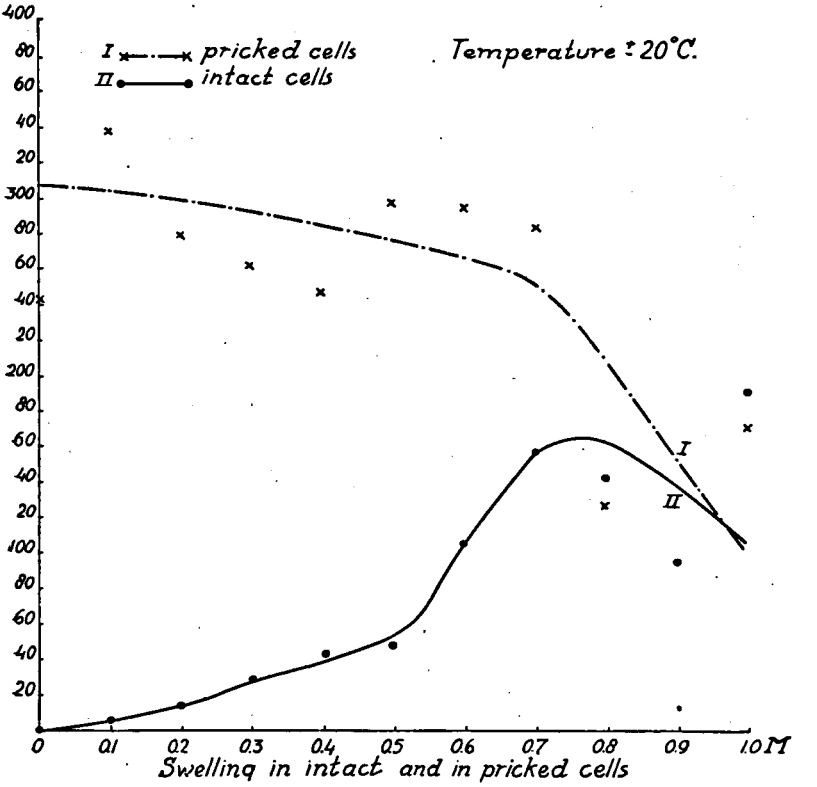
The course of the curve for the pricked cells seems very doubtful, but apart from this fact a comparison of the values in the first and in the second column shows that especially in the lower concentrations the swelling is much stronger in the pricked cells. No turgor-pressure impedes the swelling influence of the plasmolyte. The difference in the two values for each concentration below 0.8 M. is caused by the turgor-pressure. In this connection a total coincidence of the two curves above 0.8 mol. may be expected.

In the following series the influence of turgor-pressure and the swelling action of the plasmolyte were studied separately. The order of subsequent manipulations for these measurements is already described on page 303. The influence of turgor pressure in the range of concentrations is found by measuring the thickness of the

TABLE XIX.

Swelling of the wall after an hour, in percent of the original wall-thickness. Temperature ± 20° C.

M. saccharose	In pricked cells	In intact cells	Plasmolysis or not in the intact cells
0.0	242	0	Not plasmolyzed
0.1	337	7	„
0.2	277	13	„
0.3	261	30	„
0.4	246	42	„
0.5	296	48	„
0.6	294	104	„
0.7	282	157	Scarcely plasmolyzed
0.8	126	141	Plasmolyzed
0.9	159	94	„
1.0	171	190	„



Swelling in intact and in pricked cells

Graph 10.

TABLE XX (16° C.).

Effect of the concentration of saccharose-seawater solutions, expressed in percent of the original wall-thickness

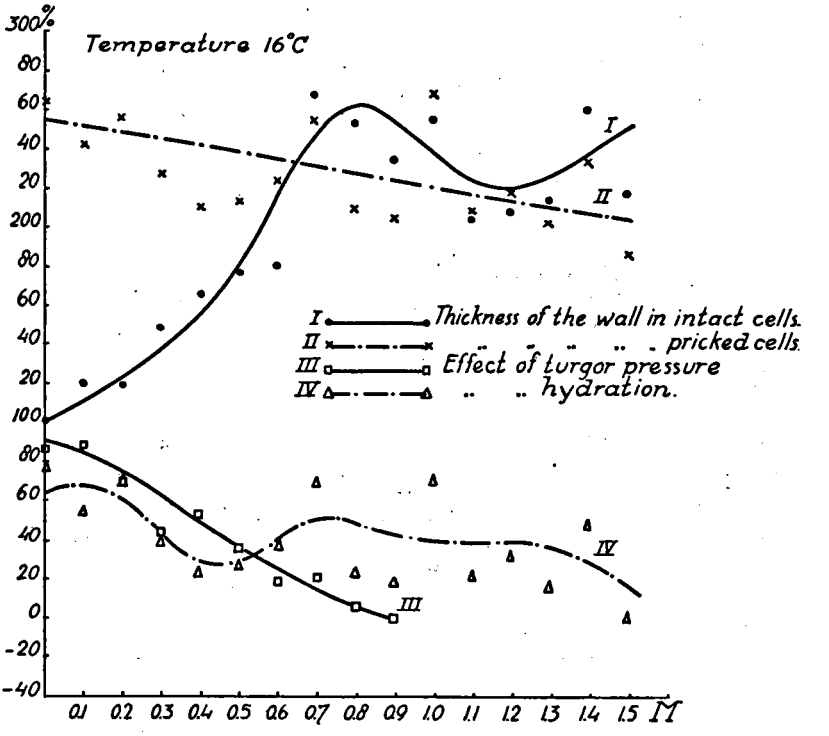
M. saccharose	I Wall-thickness in intact cells	II Wall-thickness in pricked cells	III Effect of turgor pressure	IV Effect of hydration after an hour II-186%	Number plasm. cells.
0.0	100	263	86	77	0
0.1	121	241	87	55	0
0.2	119	256	70	70	0
0.3	148	227	44	41	0
0.4	165	210	53	24	0
0.5	177	213	37	27	0
0.6	180	224	19	38	10
0.7	268	255	22	69	10
0.8	253	210	7	24	10
0.9	235	205	0	19	10
1.0	255	268		72	10
1.1	205	208		22	10
1.2	208	218		32	10
1.3	214	202		16	10
1.4	261	234		48	10
1.5	218	186		0	10

TABLE XXI (26° C.).

				II-177%	
0.0	100	254	77	77	0
0.1	118	204	71	27	0
0.2	145	210	26	33	0
0.3	151	240	58	63	0
0.4	175	228	18	51	0
0.5	169	147	6	— 30	10 —
0.6	183	208	26	31	9 =
0.7	220	200	1	23	10
0.8	206	200		23	10
0.9	204	219		42	10
1.0	213	204		27	10
1.1	179	186		9	10
1.2	189	191		14	10
1.3	219	232		55	10
1.4	184	183		6	10
1.5	198	203		26	10

— beginning plasmolysis. = plasmolysis visible.

wall after a sojourn of the cells in the plasmolyte for an hour, after which the cell is pricked and the wall measured again. The difference of these two values indicates the impeding action of the turgor

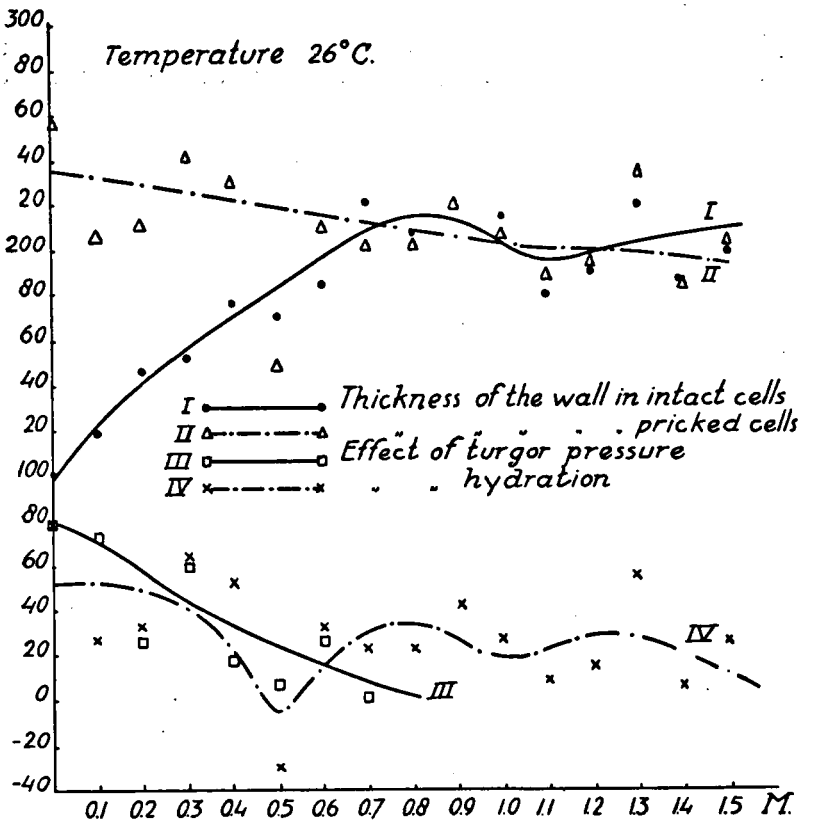


Graph 11.

pressure. The hydration value of the plasmolyte is found by reducing the swelling-value in pricked cells by the value of turgor pressure.

These two series of measurements were carried out at constant temperature, the first one at 16° C., the second at 26° C. (table XX and XXI, graph 11 and 12).

3. Microscopic measurements of length and width of the cells. Especially in the higher concentrations (1.0 M.) these measurements are anything but exact. The vacuoles are, in these concen-



Graph 12.

trations, diabolo-shaped and the thickness of the wall varies between the two transversal walls. In particular the measurement of the thickness of these transversal walls (between the vacuoles) was very difficult, as in the lower concentrations in fresh material these walls were hardly visible, and in the higher concentrations they were often cleft. Table XXII reproduces these measurements that are only mentioned for completeness sake. (See table XXII, page 326).

The mean-values of the volumes in 0.7 and in 1.0 M. saccharose-seawater solutions do not correspond with the values, found dilatometrically. Moreover, the observational error for the measurements

of length and width is 1.14 % so that a great part of the observed values are not at all real, only in the higher concentrations the values surpass the observational error, but here the measurement is much more inexact, as the vacuoles have formed 'diabolo's'.

The single conclusion we should venture to deduce from table XXII is that considering the dilatometric data, measurements in one plane are not sufficient for volume-calculations.

CHAPTER IV.

MEASUREMENTS IN DILUTED AND CONCENTRATED SEAWATER.

With the methods described in Chapter II measurements were also carried out in seawater below- and above normal concentration, these concentrations being prepared respectively by diluting the artificial seawater with distilled water and by evaporation of the artificial seawater.

§ 1. *Diluted seawater.*

In diluted seawater a swelling of the membrane could be expected, as water passing through the cell-wall is supposed to be one of the causes of swelling, for when the filaments are brought in diluted seawater it is probable that the cell contents will take in water from the surroundings. To investigate this possibility, walls in several filaments were measured after bringing these filaments in a solution of 30 % seawater and 70 % distilled water, but it was impossible to observe any membrane-swelling, as long as the cells remained intact (i.e. as long as the protoplasts are pressed against the wall). This is not astonishing, if one considers that turgor-pressure counteracts the swelling. In diluted seawater the vacuole will take in more water, so that the turgor-pressure will increase and prevent the membrane-swelling. The thickness of the wall increases only when the cell dies and the protoplast recedes from the wall.

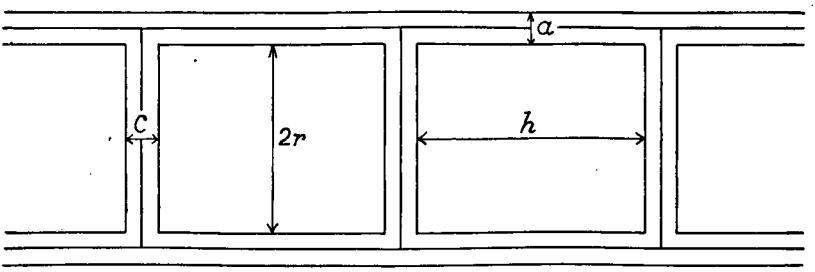
1. Refractometric measurements.

As the cells in diluted seawater are supposed to take in water from the surrounding liquid, an increase in refractive index of the liquid in the capillaries could be expected.

TABLE

Microscopic measurements

M.	Time	r	r_1	a	a_1	r + a	r_1+a_1	Change r + a	Average
0.2	15 h.	25.8	25.2	0.8	0.9	26.6	26.1	— 1.88%	
0.3	15 h.	28.4	27.95	0.98	0.93	29.4	28.9	— 1.70%	
0.4	1 h.	18.7	18.13	0.35	0.72	19.05	18.85	— 1.05%	
0.5	5 h.	30.15	29.7	0.9	1.6	31.05	31.3	+ 0.80%	
0.5	30 min.					23.45	23.31	— 0.60%	
0.5	30 min.					21.92	21.90	— 0.09%	— 0.05%
0.5	30 min.					24.64	24.77	+ 0.53%	
0.5	1 h.								
0.6	30 min.					26.25	26.18	— 0.27%	
0.6	30 min.					26.63	26.39	— 0.90%	— 0.69%
0.6	30 min.					26.96	26.73	— 0.90%	
0.6	1 h.								
0.7	30 min.					24.94	24.99	+ 0.20%	
0.7	30 min.					25.14	25.01	— 0.52%	— 0.37%
0.7	30 min.					23.14	22.96	— 0.78%	
0.7	1 h.								
0.8	1 h.								
1.0	30 min.								
1.0	1 h.								
1.0	1 h.	18.8	19.2	0.8	1.9	19.6	21.1	+ 2.55%	
1.4	1 h.	17.2	16.5	0.8	1.8	18.0	18.3	+ 1.67%	



r_1, a_1, h_1, and c, values after plasmolysis.

Table XXIII shows the observations for refractive index changes in a mixture of 70 % seawater and 30 % distilled water (n. 1.3364).

From this table it appears that, on the average, there is no change in refractive index and, therefore, no measurable quantity of water is withdrawn from the liquid in the capillary.

XXII.

of length and width.

h	h_1	c	c_1	h+c	h_1+c_1	Change h + c	Average	Change in c	Vol. after plasmolysis
41.1	40.3	0.9	0.9	42.0	41.2	— 1.90%			
44.6	44.1	1.0	0.9	45.6	45.0	— 1.32%			
32.75	31.66	0.39	0.62	32.96	32.28	— 2.06%			
50.4	48.8	0.8	1.5	51.2	50.3	— 1.76%			
				38.44	37.72	— 1.87%			
				38.66	38.86	— 0.88%	— 1.14%		
				36.32	36.08	— 0.67%			
								+113.64%	
				40.00	40.54	+ 1.35%			
				37.64	36.92	+ 1.91%	— 1.07%		
				41.28	41.26	— 0.05%			
								+78.08%	
				42.54	41.44	— 2.59%			
				42.06	41.58	— 1.14%	— 1.81%		97.4%
				33.88	33.30	— 1.71%			
								+ 52.14%	
				47.78	46.98	— 1.67%	— 1.67%	+120 %	
				44.56	42.76	— 4.04%	— 4.04%		
29.16	26.36	0.6	1.0	29.76	27.36	— 8.06%	— 8.06%		96.9%
29.0	28.15	0.8	1.3	29.8	29.45	— 1.17%	— 1.17%		

In the last column the volume after plasmolysis in 0.7 and in 1.0 M. is indicated provided that the form of the cell is still cylindrical. For 1.0 M. and upward this will not be the case.

TABLE XXIII.

Nr.	Time	n_1	n_2	Difference	
1	2 h.	1.3364	1.3368		+ 4
2	„	„	1.3368		+ 4
3	„	„	1.3366		+ 2
4	„	„	1.3361	— 3	
5	„	„	1.3363	— 1	
6	„	„	1.3361	— 3	
7	„	„	1.3363	— 1	
8	„	„	1.3361	— 3	
9	„	„	1.3363	— 1	
10	„	„	1.3363	— 1	
				— 13	+ 10

We found the same for the mixture 30 % seawater — 70 % distilled water, after two hours.

Finally the filaments were brought in distilled water, but it is evident that here no refractometric changes can be observed, as the refractive index of distilled water, also when a small quantity will be taken up in the filaments, will remain the same.

2. With the dilatometer the following results were obtained;

TABLE XXIV.

Seawater : Aq.dest.	Time	Volume-change
70 : 30	24 h.	— 0.2 %
50 : 50	12 h.	+ 0.75 %
0 : 100	3½h.	no measurable change
	8 h.	

When the filaments were left in distilled water for 24 hours they were too flaccid to be measured dilatometrically. These changes are so small that no conclusion could be arrived at.

3. Microscopic measurements.

For completeness sake some microscopic measurements in diluted seawater and distilled water are mentioned in table XXV.

TABLE XXV.

Seawater: Aq. dest.	Time	r_0+a_0	r_1+a_1	Change r + a	h_0+c_0	h_1+c_1	Change h + c
50 : 50	1 h.	24.07	24.07	0%	21.72	21.98	+ 1.20%
50 : 50	1 h.	22.76	22.70	— 0.27%	22.45	21.82	— 1.64%
50 : 50	19 h.	22.76	22.71	— 0.22%	22.45	22.53	+ 0.35%
0 : 100	1 h.	23.56	23.51	— 0.2 %	19.00	20.18	+ 6.21%
0 : 100	1 h.	21.41	21.32	— 0.42%	23.11	23.51	+ 1.73%

Only once a considerable change in length could be observed in pure distilled water, but for the rest these changes are too small to justify any conclusion.

§ 2. *Concentrated seawater.*

The observations for concentrated seawater are very few, only one set of data in seawater with a refractive index of 1.3432 (corres-

ponding to the refractive index of 0.1 M. saccharose) was obtained, the original volume being reduced to 45 % (corresponding to 0.5 M. saccharose-seawater).

It was observed that plasmolysis in this concentrated seawater recedes rapidly, due to the permeability of the protoplast. After an hour in the concentrated seawater the vacuole is already totally plasmolyzed, this was by no means the case in 0.5 M. saccharose-seawater. After two hours plasmolysis had disappeared completely. By this phenomenon the swelling-phenomena are also more or less obscured. After one hour the swelling in the longitudinal wall is 177%, when the protoplast reassumes its original position the turgor pressure increases again, and the thickness of the membrane decreases. After 20 hours the swelling of the wall proved to be 70 % of the original thickness.

1. Table XXVI demonstrates the difference in refractive index after one hour in concentrated seawater.

TABLE XXVI.

Nr.	Time	n_1	n_2	$n_1—n_2$
1	1 h.	1.3432	1.3426	6
2	„	„	1.3421	11
3	„	„	1.3426	6
4	„	„	1.3423	9
5	„	„	1.3422.	10
6	„	„	1.3421	11
7	„	„	1.3423	9
8	„	„	1.3425	7
9	„	„	1.3428	4
10	„	„	1.3422	10

Average: 8.3

In saccharose-solutions this average is reached in 0.5 M.

2. With the dilatometer a shrinkage of 1.8 % was observed after one hour.

3. Table XXVII shows the results of microscopic measurements in this concentration.

TABLE XXVII.

Time	r_0+a_0	r_1+a_1	Change r + a	h_0+c_0	h_1+c_1	Change h + c
1 h.	24.07	24.00	— 0.29%	22.03	21.52	— 2.31%
1 h.	22.58	22.55	— 0.13%	21.97	21.73	— 1.89%
4½h.	22.58	22.33	— 1.11%	21.97	22.03	+ 0.50%
19½h.	24.07	24.19	+ 0.55%	22.03	21.83	— 0.91%

CHAPTER V.

DISCUSSION OF THE RESULTS.

Chaetomorpha Linum (Müll.) Kütz., a marine Cladophorous alga, was cultured in artificial seawater in the laboratory. The cells of this alga consist of a large vacuole surrounded by a thin protoplasmic layer and a cellulose-membrane. The structure of the membrane is elucidated in a publication of Nicolai and Frey-Wyssling (1938). The longitudinal wall consists of two layers, an inner and an outer one, both containing numerous lamellae. The filaments (after removal of the plasmatic contents) show a very fine longitudinal and transversal striation. Each of the lamella contains the crossed system. Nicolai & Frey-Wyssling already showed that the greater part of the wall-substance consists of cellulose, while also pectin-like substances are present. In the way indicated by Naylor and Russell-Wells for the cell-walls of Brown-and Red Algae, we succeeded in isolating macrochemically the cellulose out of the Chaetomorpha filaments.

The potential milieu is investigated for the triple salt-combinations of NaCl, $CaCl_2$ and $MgCl_2$ and plotted in the so-called triangular diagrams after macroscopic examination of the alga. For these investigations we used a range of 10 concentrations of increasing salinity. It appeared that the lowest concentration (0.05 eq.) and the highest concentrations (1.75 and 2.0 eq.) killed the filaments at once (see fig. 2). The best conditions were found in 0.5 and 0.7 eq. the concentration of natural seawater being 0.535 eq. From these data it appeared that the environment is highly dependent upon concentration. The 'one-salt' points 0, 5 and 10 are noxious to Chaetomorpha, just as the Na-Ca-combinations and almost all of the Na-Mg-combinations. In the two-salt combinations of Ca and Mg, however, we found a range of good conditions. This fact

is so much more remarkable as these salt-combinations are not found in natural waters. The best conditions were found in the triple-salt combinations.

Another remarkable fact was that the seawater point is not the centre of the potential milieu.

The thickness of the wall and the condition of the cell was also observed microscopically (see fig. 3).

The figures in this set of triangular-diagrams show a striking correspondence with those of the first set.

From table II it appears that the wall in the intact cells (condition 0) is much thinner (0.65) than in the cells with condition 1, 2 and 3. It means that as soon as turgor-pressure decreases and the protoplast recedes from the wall, the membrane reacts by swelling, as stated already by Kotte.

After nine weeks one single filament was still alive in series IV (concentration of natural seawater) at point 16 (NaCl; 12, $CaCl_2$; 12, $MgCl_2$; 6) (Chapter I).

In the case of Chaetomorpha Linum Höfler's plasmometric method is not applicable for measuring changes in water-contents. Kotte (1914) already accentuated the absolute impossibility of an exact determination of the osmotic value in marine-algae. The swelling of the membrane in normal seawater is balanced by the turgor-pressure and retarded the recession of the protoplasm. Excessive osmotic values must be obtained in this way, as the protoplasm will only recede from the membrane when the swelling of the membrane has reached its maximum. Instead of determining the incipient plasmolysis Hoffmann (1932a) measured;

a. incipient contraction of the cells.

b. incipient membrane-swelling.

a. Also Kotte claims already that the concentration in which the incipient contraction of the cell may be observed approximates the osmotic concentration of the cell-contents. As this contraction (at least in all filamentous algae) may be easily observed, Hoffmann advocates the measurement of the cell-shrinkage instead of other methods.

b. As soon as turgor-pressure decreases (by water leaving the cell and passing into the surrounding solution), the membrane begins to swell. The concentration of the solution causing incipient membrane-swelling corresponds much better to the osmotic value of the turgescent cells than the concentration causing incipient plasmolysis, provided that the plasmolyte has no specific influence upon the membrane.

Hoffmann determined the incipient membrane-swelling by

simple microscopic observation or by measuring the lumen of the cell. The swelling of the membrane is always directed towards the centre of the cell, so that one observes the incipient swelling by a decrease of the cell-lumen, the width of the cell remaining equal.

Table XXVIII shows the correspondence between the values of incipient contraction and of incipient swelling.

TABLE XXVIII.

Name of the Alga	Concentration in G. M. saccharose	
	incipient swelling	incipient contraction
Ceramium diaphanum . . .	0.10	0.075
Callithamnium roseum . . .	0.30	0.25
Polysiphonia nigrescens . .	0.135	0.125

One of the advantages of this method is that a damage to cells caused by receding of the protoplasm may be avoided, as the concentrations used are all relatively low.

In a second article Hoffmann (1932b) draws attention to the fact that his original nomenclature ran counter to that used by Ursprung and Blum. It was not the osmotic value in the turgescent phase he measured, but the suction-pressure of the cell, and as the equation $S_z = S_i - W$ describes the relation between S_z (suction-pressure of the cell) and S_i (suction-pressure of the cell-contents), it is clear that those two values do not correspond. In the article of 1935 Hoffmann concludes that submerse algae do not have active suction-pressure, so that the values mentioned in the first article are of limited value because of the method used.

The Ursprung and Blum-method is no more applicable to such filaments as possess a membrane which plays such a large role in plasmolysis-phenomena.

The cryoscopic method is far too coarse for the study of the changes in water-contents.

In order to meet the above objections two new methods were developed (Chapter II).

By the first, refractometric, method the water-quantities, passing through the wall into the surrounding liquid, are determined, provided that only pure water leaves the cell.

In material, sent from Den Helder in May, in July and in August respectively,the water quantities excreted by the filaments in 1.17M. saccharose mutually agreed. The variability of the values in one series may probably be caused by the difference in age of the cells.

With the refractometric method four series of measurements were carried out, two at 15° C., one at ± 20° C. (room-temperature) and one at 26° C. (Chapter III).

By comparing the graphical representations of the data obtained (see graph 6), the correspondence between the four series in the region between 1.0 and 2.4 M. becomes evident. The values found at the highest temperature (26° C.) are higher than those at ± 20° C. The difference between the two curves at 15° C. may be explained by the condition of the material; curve II giving the values for material already kept in culture in the laboratory for some weeks, whereas the material used in the experiment depicted in curve I was received recently. The rectilinear relation between water-excretion and concentration at the different temperatures is clearly apparent between 1.0 and 2.2 M. (II deviates somewhat), but below 1.0 M. serious deviations from the rectilinear course occur.

While the refractometric method gives the loss of water, the dilatometric method indicates directly the 'new volume'.

From the description of the refractometric method and of the dilatometric method it appears that these methods are especially applicable to the case of Chaetomorpha. Especially the technique of the refractometric method is based upon the filamentous form of the object; for objects of other shapes the method should be modified.

Finally the swelling of the membrane was measured in the range (0—1.5 M.) of saccharose-seawater solutions in intact cells as well as in relaxed membranes. These measurements yield data on the influence of turgor-pressure and on decreasing hydration in higher concentrations as well.

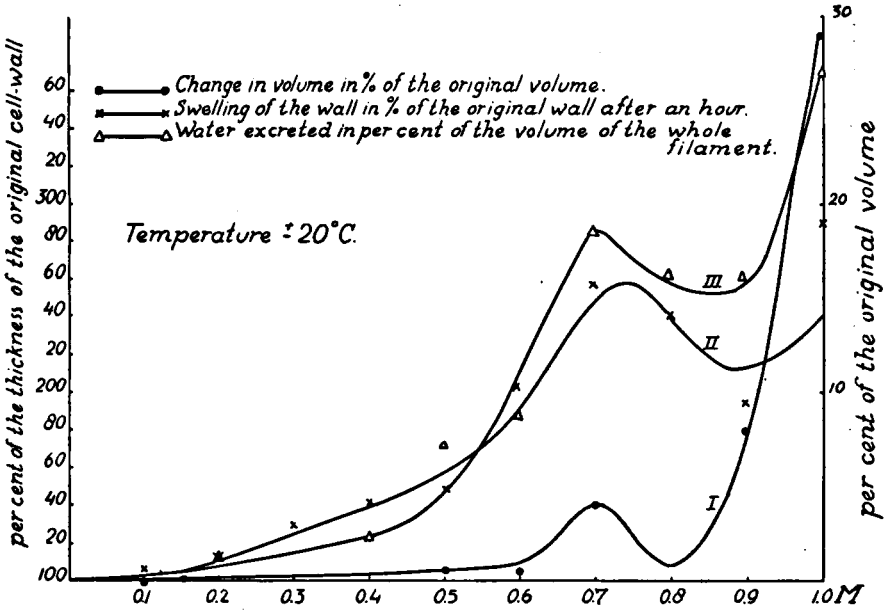
In graph 13 the data obtained by means of the three methods are plotted together [1]. The concentration of 0.7 M. appears to be a critical point. The swelling values (curve II) decrease in concentrations higher than 0.7 M. At the same time both the quantity of water passing into the surrounding solution and the shrinkage of the cell (I) decrease for which phenomenon, however, no sufficient explanation can be provided.

The same measurements were carried out in diluted and concentrated seawater (Chapter IV). In diluted seawater one should expect a considerable swelling of the membrane, as in this concentration water is passing through the cell-wall into the interior of the cell. But by the intake of water turgor-pressure increases, impeding the

[1] I wish to express my thanks to Mr. J. E. Bevelander for the care he bestowed on the figures of this paper.

swelling, and, therefore, no visible swelling of the membrane could be observed, as long as the protoplast was pressed against the cell-wall. The refractometric, as well as the dilatometric and the microscopic measurements did not yield measurable changes in water contents (except one microscopic measurement).

In concentrated seawater only one set of measurements was carried out, in a concentration corresponding to a 0.5 M. saccharose-seawater solution. The refractometric data show an equal decrease



Graph. 13.

in refraction-index as in 0.5 M. saccharose-seawater. But in the concentrated seawater the vacuole is totally contracted after an hour which is not the case in 0.5 M. saccharose-seawater, and after 3 to 4 hours plasmolysis disappears, and the swelling of the membrane decreases.

A small decrease of the volume of the filament was demonstrated by means of the dilatometric method.

Microscopic observation did not show measurable changes.

It is possible to calculate the volume of the filament after plasmolysis from the data obtained with the refractometric method

and with the microscopic measurements. It may be expected that these calculated values correspond to the values found directly by means of the dilatometric method.

The new volume measured with the dilatometric method was expressed in percent of the original volume;

M.	%
0.15	99.9
0.35	102.5
0.5	99.6
0.6	99.2
0.7	95.9
0.8	99.3
0.9	92.1

The quantity of water passing through the cell-wall into the surrounding liquid (in percent of the original volume) amounts to;

M.	%
0.15	0.6
0.35	1.9
0.5	4.8
0.6	7.5
0.7	19.5
0.8	17.1
0.9	16.0

After plasmolysis, the thickness of the wall, in percent of the original volume of the filament (provided that the filament maintains its cylindrical shape) is;

M.	%
0.15	8.5
0.35	10.4
0.5	12.1
0.6	15.9
0.7	19.1
0.8	19.3
0.9	17.4

Before plasmolysis the volume of the wall amounted to 7.8 % of the total volume of filament so that the differences between the walls before and after plasmolysis are;

M.	%
0.15	0.7
0.35	2.6
0.5	4.3
0.6	8.1
0.7	11.3
0.8	11.5
0.9	9.6

We suppose this difference to be caused by a quantity of water imbibed by the cell-wall and excreted by the vacuole, provided there is no volume-contraction. The volume of the vacuole before plasmolysis is 100 — 7.8 = 92.2 % of the original volume.

The volume of the vacuole after plasmolysis is found by reducing 92.2 % by the quantity of water excreted into the surrounding solution and by the quantity that causes the swelling of the membrane;

M.	Volume of the vacuole before plasmolysis.	Quantity of water excreted into the surrounding liquid.	Quantity of water causing membrane-swelling.	Volume of the vacuole after plasmolysis.
0.15	92.2	0.6	0.7	90.9
0.35	92.2	1.9	2.6	87.7
0.5	92.2	4.8	4.3	83.1
0.6	92.2	7.5	8.1	76.6
0.7	92.2	19.5	11.3	61.4
0.8	92.2	17.1	11.5	63.6
0.9	92.2	16.0	9.6	66.6

The volume of the whole filament after plasmolysis calculated with the data obtained by means of the refractometric method and microscopic measurements is;

M.	Volume of the vacuole after plasmolysis	Thickness of the wall after plasmolysis	Volume of the filament after plasmolysis
0.15	90.9	8.5	99.4
0.35	87.7	10.4	98.1
0.5	83.1	12.1	95.2
0.6	76.6	15.9	92.5
0.7	61.4	19.1	80.5
0.8	63.6	19.3	82.9
0.9	66.6	17.4	84.0

The differences between the calculated values and the values obtained by direct measurements are in percents of the original volume;

M.	calculated volume	measured volume	difference
0.15	99.4	99.9	— 0.5
0.35	98.1	102.5	— 4.4
0.5	95.2	99.6	— 4.4
0.6	92.5	99.2	— 6.7
0.7	80.5	95.9	—15.4
0.8	82.9	99.3	—16.4
0.9	84.0	92.1	— 8.1

An attempt will be made to explain these differences. The differences between the values calculated from water movement and those found by direct voluminometry indicate that neither intake or excretion of water proceed isometrically.

In the lower concentrations the discrepancies in the measured and calcutated values may be caused by the, however, not yet perceptible, "dead space". In higher molarities plasmolysis occurs and the negative deviations that are observed in this range also point to the absence of an isometric process which in addition to the "dead space" between wall and plasmolyzed protoplast may account for part of the discrepancies.

The relatively low discrepancy between the values at 0.9 M. may be caused by experimental errors.

Finally one has to consider that both the homogeneity of the material and the time-factor influence the results. For a fair comparison of the dilatometric, the refractometric and the microscopic data, it is necessary to apply these methods at the same time to the same material.

For obvious reasons this proved to be practically impossible.

Lack of time made it impossible to carry these investigations to completion.

This investigation was carried out at the Botanical Institute of the Government University, Leyden, Director: Prof. Dr L. G. M. Baas Becking.

I am greatly indebted to Professor Baas Becking for his inspiring ideas as to the perfection of the methods and the solving of the problem. I want to express my sincere thanks for his constant help during the years I worked under his stimulating direction.

SUMMARY.

1. Chaetomorpha Linum (Müll.) Kütz. was cultured in artificial seawater in the laboratory.

2. Cellulose, the main wall-substance, was isolated macrochemically by means of the method of Naylor and Russell-Wells (1934).

3. The potential milieu was investigated for ten successive concentrations of the triple-salt combinations of NaCl, $CaCl_2$ and $MgCl_2$ and plotted in triangular diagrams after macroscopic and after microscopic observation of the alga. A striking correspondence is found between these two sets of triangular diagrams.

4. The potential milieu appeared highly dependent upon total concentration.

5. A range of good conditions was found in the Ca-Mg combinations, although these salt-combinations do not occur in natural waters.

6. The seawater-point lay well out of the centre of the potential milieu.

7. Plasmolysis-phenomena were studied in saccharose-seawater solutions.

8. The wall in intact cells is much thinner than in cells with incipient or proceeding plasmolysis.

9. From a survey of the methods used in measuring changes in water-contents it appears that in the case of Chaetomorpha these methods are not applicable.

10. Two new methods especially adapted to the filamentous shape of Chaetomorpha are described;

a. the refractometric method.

b. the dilatometric method.

11. By means of the refractometric method the water-quantities passing through the wall into the surrounding liquid were determined. Four series of measurements were carried out, two at 15° C., one at ± 20° C. and one at 26° C.

12. With the dilatometric method the volume of the filament was measured directly after plasmolysis.

13. The swelling of the membrane was measured in intact cells as well as in pricked ones ("relaxed membranes"), in a range of saccharose-seawater solutions.

14. It was possible to calculate the volume of the filament after plasmolysis from the data obtained by means of the refractometric method and by microscopic measurement.

15. It proved to be impossible to explain quantitatively the differences between the calculated values and those obtained bij means of the dilatometric method.

16. By means of the refractometric and the dilatometric methods and microscopic measurements some data were obtained for the plasmolysis-phenomena in diluted and concentrated seawater.

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