OSMOTIC ADAPTATION OF NITELLA TRANSLUCENS Agardh.

Application of Barger's Method for determining the Osmotic Value to Vacuole Sap

by

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Contents.

A. INTRODUCTION.

The main object of my investigations was to determine the increase in the osmotic value of Nitella cells when these

are cultivated in various solutions. In the second place I investigated what the cause of this increase was — anatonosis or permeation.

For the cultivation experiments Nitella translucens Agardh. was used, which species has cells from 5 to 20 cm. long with a thickness of about 1 mm.

This Alga grows in a lake near Groningen, from which, thanks to the kindness of Prof. Dr. J. C. Schoute, I was able to obtain fresh material throughout the year. The Algae were kept in a tub filled with main-water, which was in the Botanical Garden, and in which they remained in good condition for months. At first the bottom was covered with a layer of garden-soil and sand; putrefaction, however, being found to occur, no such layer was used in 1930 and '31.

Hille Ris Lambers (1926) also used main-water for Nitella cultures; on the bottom of the tub, however, he had a layer of equal parts of clay, peat, and sand, 5 cm. thick. Osterhout (1921—'22) and Hoagland—Hibbard—Davis (1927) give recipes for culture solutions for Nitella, in which the plants may remain still longer in good condition; as, however, I had unlimited material at my disposal, I did not make use of such solutions. It is advisable to protect the tub from the light in order to prevent a too vigorous growth of algae on the Nitella cells.

The Nitellae required for the cultivation determinations were taken out of the tub and put into glass dishes about 20 cm. long and 5 cm. in width and height. These were put in a room in the laboratory; in 1931 they were further placed in a bowl of water, in order to reduce the fluctuations of temperature to a minimum.

For the determination of the osmotic values of the cells cultivated in sugar, urea, and glucose, control determinations were invariably made of cells lying in water in the same room, and sometimes of cells taken from the Botanical Garden.

The experiments were made in the years 1927 to 1931.

By „osmotic value" and „osmotic concentration" is understood, following Ursprung and Blum (1920 and 1930), the concentration expressed in mol. per litre (called n) When by means of the plasmolytic method we find that *cell sap* is isosmotic, or has the same vapour tension as a sugar or boric acid solution of a particular osmotic concentration (Barger's method), we must of course bear in mind that this value of the sap is formed by the total number of molecules and ions dissolved (Renner 1912, p. 489, Hamburger 1902, p. 14).

Except when specially mentioned, the *normal* condition, not the condition at incipient plasmolysis, is meant by the osmotic value of a Nitella cell.

B. DISCUSSION OF THE METHODS FOR THE DETERMINATION OF THE OSMOTIC VALUES OF PLANT CELLS. [1])

I. The Plasmolytic Method.

The oldest method is doubtless that of which the principle was already indicated and by which preliminary experiments were made by Hugo de Vries (1871) and Pfeffer (1877), and which the former worked out in greater detail (1877). By this method the salt or sugar solution in which plasmolysis of the cell begins to occur is determined; the solution in the cell-vacuole and of the plasmolytic are then isosmotic. This method was used not only by De Vries (see Opera e Periodicis collata), but later also by numerous

[1]) In this chapter a number of methods are not discussed, as they were for technical reasons not applicable to my Nitella investigations. There are the gravimetric method indicated and employed by Arcichovsky and others, that with the „potometer", the Toepler—Tammann „Schlierenmethode" cited by Ursprung and Blum (1926), and one or two other optical and electrical methods (see A. 1931). Equally inapplicable for my purpose was Ursprung and Blum's „lever method" (1930).

investigators, by whom it was modified and made still more accurate both for osmotic pressure and for permeability determinations. Fitting, especially, greatly increased the accuracy of the method (1915, '17, '20); for short description see also Gellhorn (1931, p. 138). For object he employed, following De Vries, cells of leaves of Rhoeo discolor. He worked with intervals of 0.0025 *n* of the plasmolysing solution, and called the solution in which at the end of half an hour half of the cellls of a slice were plasmolysed, isotonic with the vacuole sap in the cell. Fitting's method was criticized by Heusser (1917) and Stiles (1924, p. 175); Bärlund (1929) however, who improved Fitting's method still further, very rightly points out the exaggerations in Stiles' criticism.

Whatever advantages De Vries' method may possess, I did not employ it for a variety of reasons. By this method the osmotic value of the cells is found in a state in which they have just been plasmolysed. This value will frequently be higher than that of cells lying in their cultivation solution, and also in water, especially when the cells have a very elastic wall. De Vries himself showed this as early as 1877 in the illustration of a cell in a turgescent and in a plasmolytic state which has been reproduced in numerous manuals. Pantanelli (1904) also calls attention to this, whilst Eschenhagen (1889, p. 20) was also aware of it. Gratzy—Wardengg (1929, p. 311), who worked with fern prothallia, stated that this was only a minute difference, which statement is in general most certainly not correct. For this it is only necessary to follow the investigations of Ursprung and Blum (1920 and other publications). Krasnosselsky—Maximow (1925) showed that in cells of Helianthus plasmolysis did not occur until the volume of the cells had been reduced by 25 tot 30 %. See further the remarks of Höber (1926, p. 411). I found that the difference with Nitella was about 0.03 n (see p. 278).

A further drawback is that with the De Vries method the osmotic value of cells cultivated in a solution of a substance which is permeable to the protoplasm is difficult to determine. During plasmolysis the substance is very likely to flow out, with the result that too low values are obtained. That this possibility is not imaginary I found during determinations with cells which had been cultivated in urea solutions (see p. 354). Fitting (1917) also points out the danger of exosmosis with the plasmolytic method.

Fitting (1917) likewise refers to the possibility of anatonosis, the formation of osmotically active substances, during the plasmolytic determinations, as do also Zycha (1928) and Iljin. With my determinations, however, this is certainly negligible in the time necessary for plasmolysis. For the determination of incipient plasmolysis it is further essential that the cells on the outside should be absolutely clean; with Nitella, however, the formation of algae on the cells was pretty rapidly, which may render the observation of slight plasmolysis very difficult, if not impossible. With the Barger method this is of no importance, as (see below) the cell sap is sucked out and the determination of the osmotic values is carried out with it. If with the De Vries method about every half-hour a fresh (stronger) sugar solution is poured over the Nitella, in order finally to find the concentration which plasmolyses, great care is necessary to prevent the cells from being injured; this very easily occurs when they are no longer thoroughly turgescent.

A very real danger when the plasmolytic method is applied to Nitella also seems to me to be that too high a value is found, owing to the protoplasm during plasmolysis remaining too long fast to the cell wall; the markedly concave plasmolysis (which indicates great viscosity (Weber 1924, Romein 1931) points in this direction. With convex plasmolysis this factor may be apparently excluded (Hecht 1912, p. 187, see also literature cited by H.; Bärlund (1929, p. 54).

The possibility suggested by Fitting (1920, p. 169) of the plasmolytic layer close around the cells being less active than agrees with its concentration, owing to water exuding from the cells and reducing the concentration on the surface, may certainly be disregarded in view of the degree of accuracy I demand.

The Fitting method is inapplicable to Nitella if only for the fact that I should require far too much material for the cultivating tests. If, like Fitting, one has unlimited material, and if one can see at a glance whether in a slice, there is or is not plasmolysis in a great number of cells, then this is possible. With Nitella cells, which have to be closely examined one by one under the microscope to see whether they are plasmolysed, this is not feasible, as it would take far too much time, and, moreover, the last cell would be examined much later than the first one. Allowing 1 minute for the examination of each cell, and if, like Fitting, I wished to put 30 cells into each solution, using, say, 5 sugar solutions of consecutive concentrations, I should require 150 cells, whilst the last cell would be examined 2½ hours after the first one! With my cultivation-tests, which lasted for months, a number of cells would be required which would in practice be impossible to keep in good condition. More especially in sugar solutions would this be impossible, regard being had to the necessity of keeping the cells clean.

The great degree of accuracy obtained by Fitting (necessary for his permeability determinations) was unnecessary for my purpose.

II. The Method of Ursprung and Blum.

This method was first published by the two investigators in 1916, c (see also 1924) and was invariably used, if with some modifications (see e.g. 1923) in their later investigations and in those of their pupils. This method is also briefly

described by Benecke (1924, p. 56), Maximow (1929), Stiles (1924) and Höber (1926, p. 402). It is based on differences of volume in a normal state, in a water-saturated state, and in a state of incipient plasmolysis. By this the osmotic value can be determined in a normal condition. A few investigators have expressed objections to this method, such as Oppenheimer (1930), who calls attention to errors in measurement, which (regarded microscopically) may amount to as much as from 10 to 20 %. The differences in volume of the cells in a turgescent and in a relaxed condition are frequently too small to admit of conclusions being drawn from them. Furthermore, according to Oppenheimer, Ursprung and Blum's assumption that the increase in volume of a cell expanding as a result of internal pressure is proportional to the increase of pressure, is incorrect. Walter (1930) is also doubtful about the accuracy of Ursprung and Blum's experiments, and points out (1931, *b*) that swelling often renders Ursprung and Blum's measurements unreliable. In this publication (p. 330) Walter also cites Krasnosselsky—Maximow, who found that, when cells were brought from water into plasmolysing solutions, the maximum contractions of the cells were frequently found on measurement to come within the limits of error. Dixon (also cited by Walter, p. 331), likewise calls attention to the various drawbacks of the method.

With Nitella the method was found to be unsuitable, as the differences of volume of the cells in solutions of different concentration and in water were too small to be properly measured. With a cell of about 10 cm. in water, when pricked, a shortening of only 0.1 to 0.3 mm. was all that could be shown by means of an auxanometer. (When pricked, the cell attains the same volume as in a state of incipient plasmolysis). The change in thickness of various cells was not equal; moreover the thickness of a cell is not

the same at all parts, varying as much as 15 % at different points. In order to measure the diameter of a pricked cell, I pressed the cell quite flat under the microscope, whereupon it was possible to measure the half circumference ($= \pi r$) accurately to 0.1 eye piece micrometer line (1 eye piece micr. line = 34 μ), from which r and thus the diameter was calculated. The diameter measurements were taken about in the middle of the cells. If the changes in volume which the cells undergo when brought from a turgescent to a plasmolytic (pricked) condition are calculated from the changes in diameter, the small changes in length being disregarded, I found that in the case of 9 cells these varied from 5 to 46 %. This certainly shows that, principally owing to the impossibility of making accurate measurements, this method cannot be applied to Nitella. With Barger's method I found (see p. 278, tab. 14) in the case of 6 cells, which had an osmotic value of about 0.28 n, an increase in value of 0.03 n when they were brought into a state of incipient plasmolysis (i.e. about 10 %), the difference in the case of 1 cell being 0.01. This shows that the Barger method was more suitable for my purpose.

In 1930 Ursprung and Blum described still another method, the principle of which is similar to that of Barger. This method, however, is not so simple, and has disadvantages which the Barger method has not. For a discussion of this method see p. 285.

III. Höfler's Plasmolytic-volumetric Method.

For this method (1917) only one cell is necessary, an accurate determination of the volume of the contracted protoplasts in plasmolysed cells, which latter must have a geometrical shape, being required. As in the case of Nitella plasmolysis is very irregular, and the cell, owing to the slight rigidity of its walls, collapses after very little plasmolysis,

this method can be disregarded for my purpose. Prát's modification (1923) is for the same reason of equally little use.

IV. The Freezing-point-Method and Boiling-point Method.

The method by means of which the osmotic pressure is calculated from the amount the freezing point of the sap is depressed, is advocated especially by the Americans Harris, Gortner, Lawrence (1914, 1916), the Englishmen Dixon and Atkins (1910—1915), and recently especially by Walter (1928, '31, *c*, '31, *d*); it is further used by Maximow ('29). Apart from difficulties attendant on this method, the killing of plants before pressing out the sap, the influence of the pressure applied and so on (see Walter '31, *c*, Osterhout '21—'22), it should be noted that a pure vacuole liquid is not obtained by this means, but one mixed with sap from the protoplasm, the cell-wall, and possibly also from vessels. That the osmotic value in a normal state is measured in this way, as Walter (1930) says, is not quite true, although the difference seems often not to be great, as he states in his latest work „Die Hydratur der Pflanze" (in which a great deal of literature is cited!) (1931, *d*). In many cases, as is especially shown by his investigations, satifsactory results are to be obtained with it. In the publications of the writers cited by Walter in the work last mentioned (Knudson and Ginsburg '21, Von Guttenberg '27, Preising '30), who compared the osmotic value obtained by the kryoscopical- and by the plasmolytic method, these are seem to differ but slightly in many cases, but frequently also 3 or more atmospheres (approx. 0.1 *n*). That press sap is not by any means always to be considered similar to pure vacuole liquid is seen distinctly by the results of Hoagland and Davis (1923); a determination of the electrical conductivity showed that the

pressed out sap of Nitella cells was about 1.5 times as dilute as cell-sap obtained by pricking the cell and sucking out the sap. A drawback to the method is also that a good deal of liquid is required, certainly more than is to be obtained from one Nitella cell, it being thus quite impossible to determine the osmotic value of one cell. For the ordinary manner with the Beckmann thermometer at least 10 cc. of liquid is necessary, and even for the improved method of Guye and Bogdan (1903) 1.5 cc., this being also the case with the Drucker—Burian method (cited by Walter '28). With the Drucker—Schreiner microkryoscopical method (cited by Walter '31 *c*), only 0.005 cc. is required; according to Walter, however, this is less accurate and takes a long time.

The method of elevation of the boiling-point may be neglected altogether, as inaccurate results would most certainly be arrived at owing to colloid substances, flaking out of albumina, etc. in the vacuole liquid.

V. The Barger Method.

1. *The Principle.*

This method, which was originally employed for the determination of molecular weights, was first published in detail in 1904 (*a*), later again in 1915 and 1924. A short description is given by Höber (1926, p. 19).

The principle is as follows. When drops of plant sap (I worked with vacuole liquid from one cell) and of a standard solution, e.g. boric acid or cane-sugar, of known concentration, are alternately allowed to enter a glass capillary, so that between every two drops there is an air-bubble, the length of the drops of the liquid with the stronger molecular (osmotic) concentration will after a certain time become greater, and that of the drops with the smaller concentration will become smaller. For the vapour tension above the stronger solution is less than above the weaker one, with the

result that solvent will distill from the drops of the weaker solution to those of the stronger one. Yamakami (1920, *a*) demonstrated that osmose furthermore occurs through the thin layer of liquid which forms on the inside of the capillary when the drops are allowed to fall into it. Water will pass from the weaker to the stronger drops, which water, moreover, carries with it dissolved molecules. This also causes, to a still greater extent even than the distillation of solvent does, the more strongly concentrated drops to become greater and the less concentrated ones to become smaller. For a more detailed discussion of Yamakami's experiments see the summary of literature (p. 280).

If a number of capillaries are filled with drops of boric acid rising, say, by 0.02 *n* in concentration, alternated with drops of cell-sap, a tube will be found in which are e.g. drops of boric acid of 0.26 *n*, in which after a certain time the drops of cell-sap have become longer and the drops of boric acid shorter. In another tube, with, say, 0.28 *n* boric acid the drops of cell-sap will have become shorter and the boric acid drops longer. The osmotic value of the sap is then between 0.26 *n* and 0.28 *n*, and is therefore 0.27 *n*. It may, of course, sometimes happen that in one of the tubes both kinds of drops will retain their original length.

2. *The Technique. The Method applied to Nitella translucens.*

Cells of from 5 to 20 cm. in length, with a thickness of about 1 mm. were used for the determinations. A great advantage of this species is that a great deal of liquid can be obtained from one cell, ample to allow of a determination being made. From a cell 100 mm. long and 1 mm. thick, for instance, 30 cub. mm. of pure cell-sap can be obtained, from a cell 55 mm. long and 0.8 mm. thick, 12 cub. mm. [1]). From smaller

[1]) R. Collander (1930) was able to obtain from one cell of Chara ceratophylla from 10 to 15, very occasionally 40 cub. mm.

cells proportionately less liquid can be obtained than from large ones, at any rate, if absolutely pure cell-sap is desired. If one is satisfied with somewhat impure cell-sap, the quantities out of the two cells mentioned are approx. 50 cub. mm. and 20 cub. mm. respectively. The minimum quantity of sap required for one determination, calculated for 5 capillaries with a diameter of 0.3 mm., each with 3 cell-sap drops of a length of 1 mm., is about 1.1 cub. mm. It goes without saying that one must have more cell-sap at one's disposal in order to get the drops properly into the capillaries; a few cub. mm. will, however, suffice. As I had much more cell-sap at my disposal, I was able to use wider tubes (diam. approx. 0.5 mm.), into which the drops glide more easily.

With smaller species of Nitella it is necessary to add the liquid of several cells together in order to obtain a sufficient quantity; moreover there is the further disadvantage that they are much more delicate and consequently more easily injured.

The drops were measured under a microscope with a Zeiss eye piece micrometer, divided into tenths of 1 mm. The length of the drops was estimated to tenths of an eye piece micrometer line. I used a Zeiss eye piece 4, Zeiss objective A, the front lens of which was screwed off. With this system the drops were magnified 60 times, 1 line of the eye piece micrometer being equivalent to 34 μ. The accuracy with which the values can be read off is very great; I measured a drop, for instance, 20 times, and obtained the following values: 26.5, 26.5, 26.5, 26.4, 26.5, 26.5, 26.5, 26.4, 26.5, 26,5, 26.5, 26.5, 26.4, 26.4, 26.5, 26.5, 26.5, 26.5, 26.5, 26.4. The length of the drops should preferably not exceed 40 eye piece microm. lines; adjustment should be on the axis of the capillary.

When the front lens of the objective A was not screwed off, the magnification was greater, viz. 106 times, 1 eye piece microm. line = 19 μ instead of 34 μ. In this way greater

changes in length are obtained; the accuracy was found, however, to be less than with the smaller magnification. The same drop was measured 60 times both with the front-lens (magnification 106 times) and without it (magnification 60 times). Between the measurements of this drop other drops were measured, so that I should not remember the length of the drop in question, which, of course, would diminue the objectivity of the measurements. For both series of measurements I calculated the standard deviation („Streuung"). In the case of 60 measurements with the front lens $\sigma = 0.98$, without front lens 0.75. The arithmetical mean lies, therefore, in the first case between a measured length $\pm$ 2.9 (3 σ), in the second case $\pm$ 2.25. As I never measured one drop 60 times, but only once, these figures have of course no significance as absolute values, but they do indicate that the accuracy with smaller magnification (60 times) is greater than with greater magnification. The reason is probably that with stronger magnification small irregularities of the meniscus make the reading-off more uncertain; moreover, if the capillary is not perfectly horizontal in the Petridish (see p. 249), one side of the drop may be somewhat higher than the other, which with stronger magnification naturally produces greater indistinctness than with weaker magnification.

It should further be observed that measuring with stronger magnification takes longer than with weaker magnification. My measurements were therefore practically without exception made without front lens (magnification 60 times). Only if it is desired to work with intervals of 0.01 *n* is it better to use great magnification as the greater differences in length exceed the less perfect accuracy of reading-off. See tab. 3 and 4, pp. 252—257, and also tab. 5, p. 257, and the remarks on p. 256.

The collection of the cell-sap was carried out as follows. A cell is taken out of its culture solution, or, as the case

may be, out of the water, the neighbouring cells close to the test cell being cut off and the cell carefully but quickly dried with filter-paper; care must be taken that the turgescence is preserved and the concentration in the vacuole not increased by flaccidity. The cell is pricked with a capillary 10 cm. long and about 0.5 mm. thick, which has been drawn out very thin above a micro-flame. The cell-sap immediately rises in the tube as a result of capillary action, some pressure being, if necessary, exerted on the cell, but not too much, as otherwise impurities enter the tube. Care must further be taken that the liquid which comes out of the cell does not come into contact with the outside of the cell. A high degree of impurity causes a slight fall, 0.01 to 0.02 *n* of the value. Moreover it frequently causes an irregular shape to the meniscus. I made a large number of determinations for this. Pure sap was first collected according to the ordinary method, then the cell was well squeezed out, a large quantity of impurities, bubbles of protoplasm and chlorophyll granules coming out. This impure liquid had an osmotic value of 0.01 to 0.02 *n* lower than that of pure cell-sap.

If necessary, several capillaries may be filled in this way from one cell. Care must of course be taken that healthy cells are used. Criteria for this are good turgescence, a good, green colour, regular arrangement of the chloroplasts, with no hiatuses. Osterhout (1921—'22) also calls attention to these qualities.

Osterhout (1921—'22), who also required pure vacuole-liquid from Nitella for his experiments, worked in about the same way, and in this manner obtained cell-sap „quite free from protoplasm or chloroplasts"; M. M. Brooks (1922) likewise used his method. Osterhout (1921—'22) afterwards did it in a different way. With a pair of forceps he held one end of the cell fast, cut open the other end and brought this into contact with a glass slide. With another pair of forceps he gently squeezed the cell empty, so that

the cell-sap flowed on the glass slide, from which it was sucked up into a capillary. An objection to this method seems to me to be the very great chance that the liquid will become concentrated by evaporation. Irwin (1923, *a*) also cuts one end of the cell off and squeezes the cell out. My experience is that there is then a great likelihood of bubbles of protoplasm and other impurities being squeezed out. R. Collander (1930), who worked with Chara ceratophylla, cut one end of the cell off, and gently pressed the cell-sap directly into a glass capillary. Chloroplasts and solid protoplasm elements invariably came with the cell-sap, which were removed by a centrifugal machine action, or by filtering by means of filter-paper. This, of course, can only be done when one has a great deal of liquid (from a great many cells); moreover, it increases the chance of evaporation. The method used by Osterhout at the beginning of his tests, and by myself throughout, I found to be the most satisfactory one.

TABLE 1

Directly measured	After 1 day
0.29 n	0.29 n
0.28	0.28
0.28	0.27
0.26	0.27
0.28	0.27
0.26	0.26
0.26	0.27
0.26	0.27
0.27	0.26
0.27	0.27
0.26	0.25

TABLE 2

Directly measured	After 2 days	After 6 days	After 10 days
0.33 n	0.36 n		
0.27	0.28		
0.26	0.29		
0.27	0.27		
0.27	0.27		
0.30		0.32 n	
0.29		0.31	
0.31		0.34	
0.29			0.34 n
0.28			0.30
0.27			0.31

If there is no time immediately to make the determinations, the capillaries with which the cells were pricked

may be closed by means of paraffin, to prevent the sap from drying up. In tubes of this kind the vacuole liquid can be kept for a day, if care is taken that not too much air remains on both sides of the sap. The preceding two tables (tab. 1 and 2) show a number of determinations of cell-sap from various Nitella cells, measured directly after being sucked up, and after the remaining cell-sap had been kept in a closed reserve capillary for 1, 2, 6 or 10 days.

This shows that after one day practically the same osmotic value is found as directly after the collection of the cell-sap. After 2 or more days the value has risen (as a result of enzymatic decomposition?).

As a standard solution I at first used saccharose; to prevent too rapid a formation of mould I added 0.05 % $K_2 Cr O_4$ (Klebs 1887). I made a 0.5 *n* solution of the saccharose; by diluting this with the aid of a burette I obtained solutions with intervals of 0.02 *n*. These solutions were kept in stoppered flasks. I afterwards used, as did Barger, boric acid as standard solution, which is practically not dissociated (an absolute essential) and can be kept much longer than sugar. I made a solution of 0.61 *n*; at room temperature a boric acid solution cannot be made much more concentrated. In the same way as with sugar dilutions were made of this. I also used saccharose in my later determinations, when the osmotic value of the cells was greater than 0.61 *n* and boric acid cannot then be used.

I naturally had to have the certainty that cell-sap values found with saccharose solutions as a means of comparison were equal to the values found with boric acid solutions as standard solution. I found, however, that cell-sap values found with boric acid were from 0.02 to 0.03 *n* greater than when I compared sap from the same cell with saccharose. A few of the values found will serve to illustrate this.

The osmotic values of 7 different cells from different cultures was

0.31, 0.32, 0.27, 0.25, 0.26, 0.26, 0.30 *n* compared with boric acid,

0.28, 0.30, 0.24, 0.22, 0.24, 0.23, 0.28 *n* compared with saccharose,

0.03, 0.02, 0.03, 0.03, 0.02, 0.03, 0.02 *n* difference.

I then compared, by Barger's method, saccharose and boric acid directly with each other, and found that a boric acid solution showed no differences in the length of the drops if it was alternated in the capillaries with a saccharose solution which was 0.02 *n* weaker. If values above 0.30 *n* are reached, the difference becomes as great as 0.03 n. The following values demonstrate this.

boric acid:	0.26	0.28	0.28	0.27	0.30	0.30	0.31	0.32	0.25	0.32
saccharose:	0.25	0.26	0.26	0.25	0.28	0.28	0.28	0.29	0.23	0.29
difference:	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.03

Barger (1904) also compared boric acid with cane sugar, and found that 0.28 *n* sacch. had the same osmotic value as 0.295 *n* boric acid, practically the same difference, that is, as I found. Barger supposes that that difference is to be ascribed to ionization of boric acid. Boric acid, however, is practically not dissociated, and furthermore the same difference would then be obtained between boric acid and all kinds of other substances than saccharose, which is not the case. A different explanation seems to me to be more likely.

I made the solutions of boric acid and saccharose as follows: in order to obtain e.g. 1 litre of a 0.5 *n* solution, I put 0.5 gram-molecule of boric acid, or, as the case may be, of saccharose, into a standard flask of one litre, and filled this up to the volume of 1 litre. With such concentrated solutions Van 't Hoff's law no longer holds altogether good; strictly speaking, this law is valid only for infinitely diluted solutions, with which the volume taken up by the dissolved substance may be neglected. The molecular weight of saccharose being 342, the specific gravity 1.6, and the molecular

weight of boric acid 62, specific gravity 1.4, the quantity of saccharose required for 1 litre of 0.5 *n* solution is $\frac{342}{1.6} \times \frac{1}{2} = 107$ cc., that of boric acid of 0.61 *n* $\frac{62}{1.4} \times 0.61 = 27$ cc. With the sugar 893 grams of water are left in the litre standard flask, with the boric acid 973 grams. Such a 0.61 *n* boric acid solution is therefore $\frac{973}{893} = 1.1$ times as weak as a 0.5 *n* saccharose solution, that is, about 10% weaker. From the figures on p. 245 it is seen that e.g. 0.28 *n* boric acid has the same osmotic concentration as 0.26 *n* saccharose; boric acid would then act osmotically about 8 % more weakly; this can therefore be pretty well accounted for by the above calculation. With such concentrated solutions the molecular weight and the specific gravity have therefore to be taken into account.

At a much earlier date Morse (cited by Findlay 1913) noticed that with concentrated solutions the osmotic pressure observed agrees better with that calculated when one starts from the assumption that „the osmotic pressure is equal to the pressure which the substance would exercise if it existed as a gas in the volume occupied by the *solvent*, not by the *solution* as according to the Van 't Hoff theory". Höber (1926, p. 26) also calls attention to this, and Grafe (1924) also says that with greater concentration the osmotic pressure is greater than is permissible according to Van 't Hoff's theory. See further Berkeley and Hartley (1904), Frazer (cited by Renner 1912), Morse (1906, 1912, 1914) and the tables of Ursprung and Blum (1916, *c*) and of Walter (1931, *d*).

That, when the osmotic concentrations of strong solutions are being compared, not only the molecular weights, but also the specific gravity is to be taken into account, is also pointed out by Höber (1926, p. 26), Grafe (1924) and Renner (1912).

Ursprung and Blum (1930) wrongly deduce that the vapour tensions of isotonic watery solutions are inversely proportional to the specific gravities; we can only say that the *diminution* in the vapour tension has something to do with the specific gravity. The argument of Walter (1931, *a*), who wishes to refute the opinion of Ursprung and Blum, is not quite plain to me.

Höber (1926) and Grafe (1924) mention as further cause of the deviations in strongly concentrated solutions the formation of hydrates, in consequence of which part of the water is no longer able to perform its function as solvent.

In general, as Van 't Hoff (1900) remarks, his theory may be applied, provided the above is taken into account, in the case of physiological experiments in which the degree of accuracy demanded is not too great. With regard to this see also the remarks of Findlay (1913, p. 9, 1914), Nernst (1913, p. 571) and Fitting (1917, p. 569).

The possibility occurred to me that boric acid might react with substances out of the cell-sap, possibly with saccharose. That the two substances should not react in a capillary was laid down by Barger as an essential condition. Boëseken (1921) found that some hexoses (glucose, fructose) increase the electrical conductivity of boric acid and form an ester with it. I have shown (pp. 329, 331) that, at any rate in normal Nitella cells no sugars occur; as furthermore Boëseken was not able to demonstrate any influence of saccharose on boric acid, it seems to me to be out of the question that boric acid should cause any deviation of any moment whether in the case of cell-sap or in that of saccharose.

The Preparation and Filling of the Capillaries. The capillaries were drawn from bacteria-culture tubes of hard glass in a sharp gas burner (test-tubes will also do). These are first treated for a day with a solution of bichromate of potassium in concentrated sulphuric acid, then left for a day filled with distilled water, and finally rinsed with alcohol and dried; the tubes must be extremely clean. They were then drawn out, from one tube about 2 metres of capillary are obtained. The capillaries are broken off into pieces of about 8 cm. in length, care being taken that the ends are smooth, so that the tubes can be closed with the finger. The diameter I used was 0.25 to 0.75 mm., smaller than Barger used; I preferred to use capillaries of about 0.5 mm.

It is true that with smaller diameters the changes in length of the drops after the same time are smaller than with larger diameters, but less cell-sap is required (see also p. 263). The thickness of each capillary at both ends must be checked (see p. 265). I did this with a Zeiss instrument, manufactured for measuring the thickness of cover glasses. With a thickness of 0.40 mm. the difference must not exceed 0.05 mm. I used the tubes which varied too much in diameter at the two ends for sucking up the sap out of the Nitella cells. The boric acid solution is put into a small bowl, the end of the capillary is brought for an instant into contact with the surface, and about ¾ cm. of liquid is allowed to rise. The bowl is then covered so as to prevent evaporation. A tube filled with the cell-sap which is to be tested is then taken, the experimenter blows very gently on one of the ends until a drop appears on the other end. The drop must only remain outside the capillary for a very brief moment, as the concentration of such small drops when exposed to the air very rapidly increases owing to evaporation (see also Yamakami 1920, *a* and Barger 1904 *a*, p. 295). The boric acid is allowed to glide a few mm. into the capillary, if necessary the experimenter may suck at the other end or shake carefully; the damp forefinger is then pressed gently on the other end, and the protruding drop of cell-sap is then touched lightly with the end into which the boric acid was drawn; the pressure of the finger is somewhat reduced, so that a drop of cell-sap rises to a length of about 1 to 1½ mm. If a drop becomes too long, it can be shortened by holding filter-paper against the end of the capillary. The remainder of the cell-sap is then brushed off the reserve tube, and also the outside of the „drop-capillary". The drop of cell-sap is allowed to enter again a little way, a drop of boric acid of about the same length is „picked up", then again a drop of cell-sap, and so on. Three drops of cell-sap and two of boric acid of about

1 mm. were found to be sufficient. If more drops are taken, a stronger mixing of cell-sap and boric acid occurs during filling. Care should be taken, in order to prevent too strong mixing during filling, not to let the drops glide to and fro too much. On the end of the capillary a longer drop of boric acid comes again. The two long drops of boric acid are not measured, they serve merely as a seal; during the process of closing the capillary in the flame, their length usually alters irregularly. From 1 tot 1½ cm. from the ends of the large drops the capillary is closed at both ends by melting in a microflame. When one end is really quite closed, the drops are seen to move towards that end as a result of the subsequent cooling-off. Instead of being closed by melting, the ends may be closed with paraffin; I prefer melting, however, as paraffin sometimes comes off.

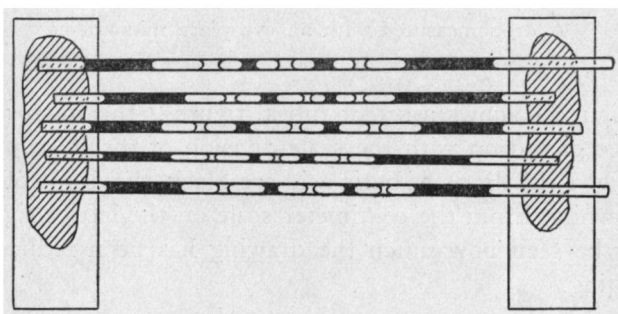

Fig. 1.

A set of capillaries according to Barger.

In this way 5 capillaries are prepared with boric acid of different strengths, and are fixed by means of vaseline or Canada balsam on to two glass strips in a Petri dish. Over these two glass strips two other strips may be fixed on to the tubes with vaseline, two prevent the capillaries from working loose. The dish is filled with water, which facilitates reading-

off with the microscope (which is placed on a table free from vibration) and checks fluctuations of temperature. It is desirable to use a microscope with a movable object-table. After about 5 to 10 minutes the drops are measured — estimation to tenths of an eye piece micrometer line. It is frequently advisable when measuring to use a small diaphragm. A lid is then placed over the dish, and 24 hours later another measurement is made.

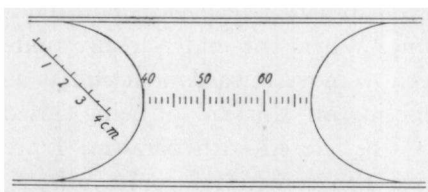

Fig. 2.
A drop measured with an eye piece micrometer.

Figure 1 shows a set of tubes, figure 2 the microscopic image of a drop with the scale-division of the micrometer. In fig. 1 the drops of boric acid are black, the drops of cell-sap white. From the centimeter scale on the left of fig. 2 it may be seen how much the drawing has been reduced in printing.

3. *Accuracy of the Method.*

I invariably worked with intervals of 0.02 *n* of the standard (boric acid) solution. Differences of 0.02 *n* are distinctly demonstrable even with cells which had an osmotic value of 0.60 *n*. If differences of 0.01 *n* are taken twice as many capillaries have to be filled and twice as many drops to be measured, the determinations thus taking so much time that the method applied to Nitella in practice loses very much of its practicability. If, for instance, we find

that the osmotic value of a cell $> 0.28\ n$ (the cell-sap drops considerably larger, the boric acid drops a good deal smaller) and $< 0.30\ n$ (cell-sap drops considerably smaller, boric acid drops larger), we give this cell a value of $0.29\ n$, which therefore means that the value lies between 0.28 and 0.30 *n*. The accuracy of the determinations is approx. the value found in this way $\pm$ 0.005 *n*. It happens, of course, that with a particular boric acid concentration no differences in length occur, e.g. with $0.29\ n$, the value is then precisely $0.29\ n$. If the extent of the osmotic concentration of a cell is absolutely unknown, a determination is first made with greater intervals, say $0.04\ n$, and if it is known that the value is between, say, 0.28 and 0.32, a new series of measurements is then made with another cell with boric acid solutions of 0.26, 0.28, 0.30, 0.32 and $0.34\ n$.

If several series of determinations are made with the cellsap of one and the same cell, the values obtained are equal. I made a number of determinations in order to ascertain whether, if so desired, it is possible to work with intervals of $0.01\ n$. Barger and many others, including myself, found that this was quite possible with purely chemical substances; with cell-sap, however, this was not certain. I carried out my measurements both with and without objective front lens, that is, with great and small magnification (cf. p. 240). The following tables give an impression of the result of the determinations in question. In all these tables the first vertical column contains the number of hours after which the length of the drops has been measured for the second time, the second horizontal row shows the length of 3 cell-sap (c.s.) and 2 boric acid drops of a concentration shown in the top column. The third horizontal row shows the length of the drops after the time stated in the first vertical column, whilst the bottom row of figures indicates the difference in length of the drops after that particular time. The lengths are estimated in tenths of the eye piece micrometer lines.

TABLE 3*a*

After	cs	0.29	cs	0.29	cs
	48.0	26.9	28.0	32.2	45.0
23 hours	48.1	26.9	28.1	32.0	45.4
Difference	+ 0.1	0	+ 0.1	— 0.2	+ 0.4

Total difference cell sap drops (cs): + 0.6
„ „ 0.29 *n* bor. ac. drops: — 0.2
Value cs > 0.29 *n*

The diameter of the four tubes of the four tables 3*a, b, c, d* is about 0.35 mm. The measurement was performed without a front lens of the objective (magnification

TABLE 3*b*

After	cs	0.30	cs	0.30	cs
	44.2	39.5	33.1	36.1	22.1
23 hours	44.4	39.5	33.6	36.2	22.4
Difference	+ 0.2	0	+ 0.5	+ 0.1	+ 0.3

Total difference cs: + 1.0
„ „ 0.30: + 0.1
Value cs > 0.30 *n*

60 times). The osmotic value of the cell-sap is 0.31 *n*. Intervals of 0.01 *n* of the boric acid are found to be possible.

TABLE 3*c*

After	cs	0.31	cs	0.31	cs
	34.4	23.0	29.2	24.9	21.9
23 hours	34.1	23.1	29.2	24.9	22.4
Difference	— 0.3	+ 0.1	0	0	+ 0.3

Total difference cs: 0
„ „ 0.31: + 0.1
Value cs = 0.31 *n*

TABLE 3*d*

After	cs	0.32	cs	0.32	cs
	11.6	24.3	27.4	16.0	16.1
23 hours	11.5	24.6	27.4	16.3	15.7
Difference	— 0.1	+ 0.3	0	+ 0.3	— 0.4

Total difference cs: — 0.5
„ „ 0.32: + 0.6
Value cs < 0.32 *n*

The four tables 3 A, B, C, D indicate the lengths of the same drops as in the tables 3*a, b, c, d* this time, however, with a magnification of 106 times (*with* front lens of

TABLE 3A

After	cs	0.29	cs	0.29	cs
	—	50.2	52.8	60.7	—
23 hours	—	50.2	53.0	60.2	—
Difference.....		0	+ 0.2	— 0.5	

Total difference cell sap drops (cs): + 0.2
„ „ 0.29 *n* bor. ac. drops: — 0.5
Value cs > 0.29 *n*

the objective), the differences in length with great magnification being greater than with small magnification; tables

TABLE 3B

After	cs	0.30	cs	0.30	cs
	83.5	74.2	62.9	68.3	42.0
23 hours	83.8	74.2	63.4	68.4	42.4
Difference	+ 0.3	0	+ 0.5	+ 0.1	+ 0.4

Total difference cs: + 1.2
„ „ 0.30: + 0.1
Value cs > 0.30 *n*

TABLE 3C

After	cs	0.31	cs	0.31	cs
	65.0	43.5	55.0	46.2	41.1
24 hours	64.4	43.6	55.0	46.1	41.7
Difference	— 0.6	+ 0.1	0	— 0.1	+ 0.6

Total difference cs: 0
„ „ 0.31: 0
Value cs = 0.31 *n*

3*a, b, c, d* however, show that it is here possible to measure with an accuracy of 0.01 *n* even with small magnification.

TABLE 3D

After	cs	0.32	cs	0.32	cs
	21.7	45.9	52.0	30.1	30.1
24 hours	21.7	46.4	51.9	30.8	29.2
Difference	0	+ 0.5	— 0.1	+ 0.7	— 0.9

Total difference cs: — 1.0
„ „ 0.32: + 1.2
Value cs < 0.32

The diameter of the four tubes of the tables 4 (cell cultivated in water) is about 0.5 mm. Measurement in tables 4*a, b, c, d* without front lens (magnification 60 times). With

TABLE 4*a*

After	cs	0.28	cs	0.28	cs
	37.0	21.9	34.5	28.0	30.1
24 hours	37.2	21.8	34.8	28.0	30.4
Difference	+ 0.2	— 0.1	+ 0.3	0	+ 0.3

Total difference cell sap drops (cs): + 0.8
„ „ 0.28 *n* bor. ac. drops: — 0.1
Value cs > 0.28 *n*

TABLE 4*b*

After	cs	0.29	cs	0.29	cs
	21.9	30.0	23.0	24.9	23.9
24 hours	22.0	29.9	23.1	24.8	23.6
Difference	+ 0.1	— 0.1	+ 0.1	— 0.1	— 0.3

Total difference cs: — 0.1
„ „ 0.29: — 0.2
Value cs = 0.29 *n*

TABLE 4*c*

After	cs	0.30	cs	0.30	cs
	24.8	28.9	24.0	29.1	23.9
24 hours	24.8	29.0	24.0	29.1	23.9
Difference	0	+ 0.1	0	0	0

Total difference cs: 0
„ „ 0.30: + 0.1
Value cs = 0.30 *n*

TABLE 4*d*

After	cs	0.31	cs	0.31	cs
	24.4	14.1	35.4	31.6	39.3
24 hours	24.0	14.5	35.2	32.0	39.0
Difference	— 0.4	+ 0.4	— 0.2	+ 0.4	— 0.3

Total difference cs: — 0.9
„ „ 0.31: + 0.8
Value cs < 0.31 *n*

intervals of 0.01 *n* this magnification is with this series of determinations too small, no changes in the drops occurring either with 0.29 or with 0.30 *n* boric acid. This may be due e.g. to too strong mixing of the drops during the filling of the capillaries.

TABLE 4A

After	cs	0.28	cs	0.28	cs
	70.0	40.9	65.0	52.9	57.0
24 hours	70.3	40.8	65.8	52.8	57.4
Difference	+ 0.3	— 0.1	+ 0.8	— 0.1	+ 0.4

Total difference cell sap drops (cs): + 1.5
„ „ 0.28 *n* bor. ac. drops: — 0.2
Value cs > 0.28 *n*

The tables 4 A, B, C, D show the same drops as in the tables 4*a, b, c, d* but this time with a magnification of 106 times. From these it is distinctly seen that the osmotic value

TABLE 4B

After	cs	0.29	cs	0.29	cs
	40.9	56.0	43.1	46.7	44.3
24 hours	40.9	55.8	43.4	46.4	44.1
Difference	0	— 0.2	+ 0.3	— 0.3	— 0.2

Total difference cs: + 0.1
„ „ 0.29: — 0.5
Value cs > 0.29 *n*

of the cell-sap is > 0.29 *n* and < 0.30 *n*. In this case (tables 4*a, b, c, d* and 4 A, B, C, D it is thus still possible to obtain

TABLE 4C

After	cs	0.30	cs	0.30	cs
	48.1	54.1	45.2	54.9	44.8
24 hours	48.0	54.4	45.0	55.1	45.0
Difference	— 0.1	+ 0.3	+ 0.2	+ 0.2	+ 0.2

Total difference cs: — 0.1
„ „ 0.30: + 0.5
Value cs < 0.30 *n*

TABLE 4D

After	cs	0.31	cs	0.31	cs
	45.9	26.2	66.3	59.1	74.3
24 hours	45.3	27.0	66.2	60.0	73.7
Difference	— 0.6	+ 0.8	— 0.1	+ 0.9	— 0.6

Total difference cs: — 1.3
„ „ 0.31: + 1.7
Value cs < 0.31 *n*

an accuracy of 0.01 *n* with the great magnification (tab. 4 A, B, C, D) but with small magnification one has to be content with intervals of 0.02 *n*.

TABLE 5*a*

After	cs	0.52	cs	0.52	cs
	19.4	14.0	16.2	11.9	39.0
24 hours	22.6	12.0	18.7	9.5	42.0
Difference	+ 3.2	— 2.0	+ 2.5	— 2.4	+ 3.0

Total difference cell sap drops (cs): + 8.7
„ „ 0.52 *n* bor. ac. drops: — 4.4
Value cs > 0.52 *n*

In the tables 5 (a cell which was cultivated in saccharose) the measurements are shown with a magnification of 60

TABLE 5*b*

After	cs	0.53	cs	0.53	cs
	9.0	23.2	12.9	12.9	11.8
24 hours	9.1	23.2	12.9	12.8	11.9
Difference	+ 0.1	0	0	— 0.1	+ 0.1

Total difference cs: + 0.2
„ „ 0.53: — 0.1
Value cs = 0.53 *n*

TABLE 5c

After	cs	0.54	cs	0.54	cs
	13.9	18.9	47.0	26.5	19.3
24 hours	13.8	18.9	47.2	26.2	19.1
Difference	— 0.1	0	+ 0.2	— 0.3	— 0.2

Total difference cs: — 0.1
„ „ 0.54: — 0.3
Value cs = 0.54 *n*

times; it is seen that, with a value of approx. 0.53 *n*, at any rate with small magnification, intervals of 0.01 *n* are too small; for we find that the osm. val. = 0.53 *n* and = 0.54 *n*.

TABLE 5d

After	cs	0.55	cs	0.55	cs
	32.0	17.5	15.0	15.0	21.2
24 hours	31.7	17.9	14.7	15.6	20.9
Difference	— 0.3	+ 0.4	— 0.3	+ 0.6	— 0.3

Total difference cs: — 0.9 } 1.9
„ „ 0.55: + 1.0 }
Value cs < 0.55 *n*

It is, however, possible to work here with an interval of 0.02 *n*.

TABLE 5e

After	cs	0.56	cs	0.56	cs
	15.4	14.9	16.3	23.0	15.4
24 hours	15.2	15.0	16.0	23.0	15.2
Difference	— 0.2	+ 0.1	— 0.3	0	—0.2

Total difference cs: — 0.7 } 0.8
„ „ 0.56: + 0.1 }
Value cs < 0.56 *n*

TABLE 5*f*

After	cs	0.57	cs	0.57	cs
	20.2	11.9	14.3	26.7	32.6
24 hours	20.0	20.3	14.2	26.9	32.1
Difference	—0.2	+ 8.4	— 0.1	+ 0.2	— 0.5

Total difference cs: — 0.8 } 9.4
„ „ 0.57: + 8.6 }
Value cs < 0.57 *n*

From tables 3*a, b, c, d* and 3 A, B, C, D, 4*a, b, c, d* and 4 A, B, C, D, and 5, we may therefore conclude that an interval of 0.01 *n* is sometimes possible with small magnification in the case of cell-sap of an osmotic value of approx. 0.30 *n* (tab. 3*a, b, c, d*) and sometimes not (tab. 4*a, b, c, d,* 5).

With great magnification *it is possible, at least with values of about 0.30 n, to use an interval of 0.01 n in the boric acid solutions.* Even if the accuracy of the length-measurements with great magnification is less than with small magnification (see p. 241), owing to the greater differences in length, it is possible, after 24 hours, at any rate with values of 0.30 *n*, to work with intervals of 0.01 *n*, which with small magnification cannot always be done. As I practically always worked with intervals of 0.02 *n* (for reasons see p. 241), I usually measured with small magnification, as this is quicker.

A number of tables may further serve to give an idea of the differences in length of the drops with divergent values of the cell-sap. Intervals of 0.02 *n*. All measured with small magnification.

These two tables (6) (cell from water) also illustrate what thin tubes may be used (0.2 to 0.3 mm.). The value of thts cell is 0.29 *n*.

TABLE 6*a*

After	cs	0.28	cs	0.28	cs
	14.0	14.0	16.8	21.7	24.2
17 hours	14.2	14.0	16.9	21.1	24.9
Difference	+ 0.2	0	+ 0.1	— 0.6	+ 0.7

Total difference cs: + 1.0
„ „ 0.28: — 0.6
Value cs > 0.28 *n*
Diameter (internal) 0.3 mm.

From the tables 7 (a cell which has been in 0.10 *n* saccharose for 41 days) it will be seen that determinations may be made with a value of 0.46 *n* with an interval of 0.02 *n*.

TABLE 6*b*

After	cs	0.30	cs	0.30	cs
	26.9	33.8	52.1	33.9	29.2
17 hours	26.9	34.1	52.0	34.2	29.0
Difference	0	+ 0.3	— 0.1	+ 0.3	— 0.2

Total difference cs: — 0.3
„ „ 0.30: + 0.6
Value cs < 0.30 *n*
Diameter (internal) 0.23 mm.

Tables 8 (normal cell cultivated in the dark) show that it is still possible to make determinations with an interval of 0.02 *n* with a value of 0.59 *n*.

TABLE 7*a*

After	cs	0.44	cs	0.44	cs
	21.3	19.7	15.5	29.9	7.5
24 hours	22.0	19.4	15.9	29.1	7.5
Difference	+ 0.7	— 0.3	+ 0.4	— 0.8	0

Value cs > 0.44 *n*

TABLE 7*b*

After	cs	0.46	cs	0.46	cs
	50.1	33.1	7.9	8.1	45.7
24 hours	50.0	33.0	7.9	8.3	45.8
Difference	— 0.1	— 0.1	0	+ 0.2	+ 0.1

Value cs = 0.46 *n*

From the last tables (6, 7, 8) and also from the preceding ones (3, 4, 5) it is seen determinations can always be made with small magnification with intervals of 0.02 *n*.

The fact that the total shortening of the drops with the

TABLE 7*c*

After	cs	0.48	cs	0.48	cs
	51.1	37.4	26.9	38.4	11.5
24 hours	50.9	37.9	26.4	39.0	11.0
Difference	— 0.2	+ 0.5	— 0.5	+ 0.6	— 0.5

Value cs < 0.48 *n*

smallest concentration is very frequently less than the total lengthening of the drops with the greatest concentration will be dealt with later (p. 266).

TABLE 8*a*

After	cs	0.58	cs	0.58	cs
	14.2	10.0	17.1	4.7	18.2
24 hours	14.4	9.9	17.4	4.8	18.5
Difference	+ 0.2	— 0.1	+ 0.3	+ 0.1	+ 03.

Total difference cell sap drops : + 0.8
„ „ 0.58 *n* bor. ac. drops: 0
Value cs > 0.58 *n*

TABLE 8*b*

After	cs	0.60	cs	0.60	cs
	12.9	18.6	21.1	10.8	35.2
24 hours	12.9	18.9	20.9	11.0	35.2
Difference	0	+ 0.3	— 0.2	+ 0.2	0

Total difference cs: — 0.2
„ „ 0.60: + 0.5
Value cs < 0.60 *n*

4. *Factors which affect the Accuracy.*

There are several factors which determine the nature and the size of the changes in length of the drops. I investigated these principally by means of determinations with various saccharose concentrations. Barger (1904) has also made a partial study of the various factors.

a. The Difference of Concentration of the two Kinds of Drops in the Capillary.

The changes in length of the two kinds of drops will naturally be greater if the concentration difference is e.g. 0.1 *n* than if it is e.g. 0.02 *n*; the greater the difference in concentration, the greater also the changes. I think it superfluous to give instances of this. For these see Barger (1904, *a*).

b. The Mixing of the Drops during filling.

In practice this is, of course, never entirely avoidable. Barger (1904, *a*) concludes on theoretical grounds that this takes place least when the drops enter the tube for the shortest possible distance; care must especially be taken that they do not glide backwards and forwards. The drops must be as close together as possible (a more rapid interchange of the vapour tensions will then, of course, occur, and osmosis will then be able to take place more rapidly in the thin layer of liquid on the inside wall (see p. 239).

Furthermore, the length of the drops must be as great as possible, and also the diameter of the capillary; it must, of course, also be possible for a good meniscus to form. It goes without saying that, if the two latter conditions are complied with, more liquid will be required than if the drops are shorter and the diameter of the capillary smaller. As I was only able to get comparatively little cell-sap out of one Nitella cell, I had to compromise somewhat. I used capillaries with a smaller diameter (0.3—0.5 mm.) than did Barger (1.5 mm.). It is, moreover, obvious that the more drops are allowed to enter the tube, the stronger will be the mixing. Barger was aware of this, and therefore always took 5 drops, 3 of one solution and 2 of the other (boric acid), an example which I followed.

I checked the accuracy of these influences, as reasoned out by Barger:

c. Influence of the Diameter of the Capillary.

In order to determine this the length of the drops and the distances between the drops in the capillaries must be as far as possible equal. It made no difference whether I had end-drops of 0.25 *n* or of 0.20 *n*.

Saccharose solutions of 0.20 *n* and 0.25 *n* were compared with one another. The tables show not only the length of the drops (under „0.20" and „0.25"), but also the length of the air-bubbles between them (Air). These, like the drops, were kept as far as possible of equal length.

The length of the drops in the capillary of 0.64 mm. diameter was about 21 micrometer lines, that of the air-bubbles between the 5 drops to be measured averaged 45 lines; in the capillary with a diameter of 0.32 mm. respectively 24 and 46 microm. lines.

In the capillary with the diameter 0.64 mm. the total length of the 5 drops was more than twice as great as in the capillary with the diameter 0.32 mm.: 8.3 and 3.7 lines respectively.

TABLE 9*a*

Diameter 0.64 mM. Zeiss eye piece 2, objective A without frontlens,

After	Air	Enddrop 0.20	Air	0.25	Air	0.20	Air	0.25	Air	0.20	Air	0.25	Air	Enddrop 0.20	Air
	27.5 mm.	5.8 mm.	50.5	21.0	48.4	15.0	33.6	21.0	49.0	22.4	47.1	24.3	41.4	6.7 mm.	27 mm.
24 hours.		5.7 mm.	48.8	22.3	47.1	14.0	32.7	23.9	47.2	21.9	45.4	27.0	39.0		
Differ. ..				+ 1.3		— 1.0		+ 2.9		— 0.5		+ 2.7			

Total difference of the 0.25 *n* drops: + 6.9 } 8.4
„ „ of the 0.20 *n* drops: — 1.5 }

TABLE 9*b*

Diameter 0.32 mM.

After	Air	Enddrop 0.20	Air	0.25	Air	0.20	Air	0.25	Air	0.20	Air	0.25	Air	Enddrop 0.20	Air
	25 mm.	7.9 mm.	69.6	23.9	46.6	21.3	47.4	30.7	36.2	27.3	37.0	19.2	41.7	8.0 mm.	20 mm.
24 hours.			57.5	24.2	46.8	21.0	46.8	31.7	36.2	27.2	35.4	21.2	39.3		
Differ. ..				+ 0.3		— 0.3		+ 1.0		— 0.1		+ 2.0			

Total difference 0.25 *n*: + 3.3 } 3.7
„ „ 0.20 *n*: — 0.4 }

d. Influence of the Length of the Drops.

In these determinations the diameter of the two capillaries in which were put 5 drops of saccharose 0.20 and 0.25 *n* alternatively was 0.50 mm., the lengths of the air-bubbles were also kept as nearly alike as possible (59 and 54 lines respectively); the drop-length in the first capillary was about 60 lines, in the second one 12 micrometer lines. The two tables which were arranged like table 9 and which are not given here show that in the first case the total change in length of the drops (9.4 lines) is much greater than in the second (2.7 lines).

e. Influence of the Length of the Air interspaces.

The determinations and tables (not given here) were arranged as described under *c* and shown in table 9. In this case however the diameter of the two capillaries is equal (0.40 mm), and also the droplengths (37 and 35 micrometer lines respectively). In the first capillary the length of the aire bubbles was about 180 lines, in the second capillary 36 lines; in the first case the change in length of the drops (3.6 lines) was much smaller than in the second case (9.1 lines).

f. Difference in Diameter at the two Ends of the Capillary.

If drops of some solution or of water are allowed to enter a capillary which is of equal width along its entire length, all the drops will, after a certain time, have become smaller through evaporation in the air-spaces between the drops. With regard to a factor which tends to neutralize this diminution see p. 266. If the diameters at the two ends differ, then all the drops will move as a result of capillary action to the narrower end, which would cause elongation and neutralize the shortening due to evaporation. I found that when the

diameters differed 50 %—100 % the changes in length become irregular; if the difference is less, very few irregularities are found. In a tube of which the diameters at the two ends were 0.60 and 0.40 mm., and into which I put 4 drops of 0.28 *n* and 3 of 0.26 *n* alternately, the total changes in length were 1.9 lines for 0.28 *n* sugar after 24 hours, — 0.6 for 0.26 *n* sugar. In another tube with a diameter of 0.30 and 0.50 mm. these changes were —1.9 for 4 drops of 0.26 *n* and 0 for the 3 drops of 0.28 *n*. For safety's sake I was always careful not to have the difference in diameter greater than about 15 %. Tubes with a greater difference were used as reserve capillaries to suck cell-sap up into.

5. *Irregularities in the Changes in Length.*

It has already more than once been pointed out in connection with the tables that the decrease in the case of the drops with the smaller concentration is generally less than the increase of the drops with the stronger concentration, regard being had to the fact that there were 2 of one kind of drops and 3 of the other. For this see practically all the tables so far given. This is often so much so that even the drops with the smallest concentration showed an increase, which increase was, however, always smaller than that of the drops with the largest concentration (see e.g. tab. 3*b* and 3 B p. 252, 253). Barger (1904, *a* p. 293) also noted this and tried to explain the phenomenon in the following way. He supposed that, during filling, small drops remained clinging to the inside wall between the drops which were to be measured, these drops being convex and having therefore a greater vapour tension than the large drops which have a concave meniscus. These convex little drops will therefore evaporate and be deposited on the large concave drops, which will thus all have a tendency to increase in size. If a comparison is made of drops in one capillary which differ but little in concentration, this phenomenon will decrease the sensitive-

ness of the method to an inconvenient extent. Care must be taken that as little mixing as possible takes place during filling (the drops being prevented as far as possible from sliding backwards and forwards, so that the possibly already small difference in concentration does not become still smaller.

An instance with Nitella sap may be given here (table 10).

TABLE 10

After	cs	0.24	cs	0.24	cs
	19.0	23.3	30.1	19.1	36.1
24 hours	21.0	23.9	31.0	19.1	38.5
Difference	+ 2.0	+ 0.6	+ 0.9	0	+ 2.4

Total difference of the cellsap drops (cs) : + 5.3
„ „ of the sacch. 0.24 *n* drops: + 0.6

From this the conclusion may safely be drawn that the osmotic value is $> 0.24\ n$.

With regard to the cause of the above-mentioned phenomenon, I further thought of a possible influence of the large end-drops, which were not measured. From a series of determinations made with these, I found, however, that the change in the large drops, whether they had the greater concentration of the two kinds of drops in the tube or the smaller, had no connection whatever in any respect with the changes in length of the small drops. The changes in the large drops were, as Barger had pointed out, very irregular.

The cause of the above-mentioned phenomenon is probably to be found in the evaporation of small convex drops, the more so as I was sometimes able to see these microscopically. It should, however, be pointed out that this does not entirely agree with Yamakami's experiments (1920, *a*) (see pp. 239 and 280), with the results of which Barger (1924) agrees. For this see further p. 280.

The time after which the drops are to be measured for the second time should preferably not be less than 24 hours. With a longer interval the differences in length often become no more distinct, whilst it further not infrequently happens that after 48 hours or longer all the drops become very considerably longer, whereas after 24 hours the drops with the smaller concentration become shorter or at any rate much less longer, than the drops with the greater concentration. An equilibrium only comes about after a very long time, as was also observed by Barger.

On p. 299 (1904, *a*) Barger says that, if the change in the length of the drops of a solution with respect to one concentration of the standard solution is known, it is not possible *to calculate* the concentration of that solution the strength of which is unknown. This is not even possible if its behaviour with respect to two known concentrations is known and if all the conditions (length of drops, diameter, etc.) are as far as possible alike. Ursprung and Blum (1930 p. 290), who describe a method which is very like Barger's (see p. 236 and in greater detail p. 285), also point out that the graphs of the relations between the size of the drops and the osmotic value are not a straight line, but have an irregular course, which is caused by irregularities in the changes in the length of the drops. Various factors (filling, diameter, etc.), it is true, do not alter the nature of the changes in length, but they do affect the absolute size. But even when all the conditions were as far as possible alike, Barger frequently found irregular changes to take place. And yet Barger interpolates (1915); if, for instance, he finds (Barger p. 5) that a particular solution is stronger than 0.13 *n* standard solution (total change in the length of the drops 16) and weaker than 0.14 (tot. change 7 lines), the value is then, according to Barger $0.13 + \frac{16}{16+7} \times 0.01 = 0.137\,n$.

The irregularities above mentioned are also clearly

to be seen in my tables 3 A, B, C, D (p. 253); the value of the cell-sap is 0.31 *n*. The total difference of the changes in length with 0.30 *n* = 1.3 (tab. 3 B), with 0.29 *n* 0.7 (tab. 3 A), although it would be expected that the difference in that case would be greater than 1.3. A still more striking example is tab. 5 (p. 258); the value is between 0.52 and 0.55. With 0.55 the total change is 1.9 (tab. 5 d), with 0.56 0.8 (tab. 5e), with 0.57 it is again larger, 1.4, but less, that is, than with 0.55. If all these changes were regular, one would naturally expect a greater change according as one gets farther away from the value of the cell-liquid. We cannot, therefore, regard the absolute change, in view of the possibility of interpolation as a criterion, but only from the nature of the changes (decrease or increase of the length of the two kinds of drops. With regard to irregularities see also Yamakami's experiments described on p. 280.

In his publication of 1904 Barger does not interpolate; it is strange, however, to note that he does so in 1915 and even cites as an instance a case of glucose and saccharose of which he gives the table in 1904, but then without interpolation. *Interpolation is to my mind never permissible.*

If it is *desirable* when working with pure chemical substances to make the second measurement not later than 24 hours after the first, it is *essential* not to wait longer than 1½ days when the osmotic value of the cell-sap is to be determined. The following was found to be very frequently the case. If after 24 hours a value of, say, 0.26 *n* is found for cell-sap, this may, when the same drops are again measured a few days later, have changed into < 0.26 *n*. I first noticed this with saccharose as standard solution.

I then tried to find out whether the same thing occurred when I used other substances than cane sugar for the standard solutions. I found this inversion both with glucose, urea, and boric acid. An instance may be given here with boric acid (table 11).

TABLE 11

Date	cs	0.30	cs	0.30	cs	0.30	cs	
25–VI–29, 16 h....	16.0	19.0	9.2	15.4	21.6	18.1	8.5	
26–VI–29, 16 h....	16.2	18.9	9.5	15.2	21.9	18.0	8.8	
Difference	+ 0.2	- 0.1	+ 0.3	- 0.2	+ 0.3	- 0.1	+ 0.3	tot. diff. cs: + 1.1; 0.30: — 0.4; value cs > 0.30 *n*
29–VI–29, 17 h....	16.0	19.8	9.0	16.0	22.0	18.9	8.2	
Differ. with 25–VI	0	+ 0.8	- 0.2	+ 0.6	+ 0.4	+ 0.8	- 0.3	tot. diff. cs: - 0.1; 0.30: + 2.2; value cs < 0.30 *n*

After 1 day we find a value of $> 0.30\,n$, after 4 days $< 0.30\,n$. It might be thought that there was a conglomeration of molecules in the cell-sap. Remarkably enough, however, I found that the value remains constant or increases, when, after having made a determination of the cell-sap in the manner described on p. 243, I kept some of the same liquid in a reserve capillary and made another determination with this a few days later (tab. 1 and 2, p. 243). A determination was now made of a number of cells and the measurement repeated a few days later (as in tab. 11); the value was then frequently found to be *lower* (tab. 12 under A). Some cell-sap of the same cell was also kept in a spare capillary, and the value of this again determined after a few days; this was then generally found to be *greater* (cf. pp. 243 and 244). These values are given in tab. 12 under B.

As the lowering of the value of the cell-sap was observed with such divergent substances for the standard solutions as saccharose, glucose, boric acid, and urea, it is difficult to account for the phenomenon by assuming a chemical influence which these substances may be supposed to have on the cell-sap.

TABLE 12

First measurement	After 2 days		After 6 days		After 10 days	
	A	B	A	B	A	B
0.27*n*	0.28*n*	0.28*n*	—	—	—	—
0.26	0.24	0.29	—	—	—	—
0.27	0.27	0.27	—	—	—	—
0.27	0.26	0.27	—	—	—	—
0.33	0.33	0.36	—	—	—	—
0.30	—	—	0.29*n*	0.32*n*	—	—
0.29	—	—	0.26	0.31	—	—
0.31	—	—	0.31	0.34	—	—
0.29	—	—	—	—	0.27*n*	0.34*n*
0.28	—	—	—	—	0.24	0.30
0.27	—	—	—	—	0.26	0.31

When I compared two concentrations of the same solution in a tube, I invariably found, even after more than one day, that the length of the drops with the smaller concentration decreased and that of the greater increased. When I compared saccharose of, say, 0.20 *n* with glucose, urea, or boric acid of stronger concentration, e.g. 0.22 *n*, then the length of the saccharose drops, as was to be expected, decreased after 1 day, while that of the others increased. Sometimes, however, I found that after several days the total change in length of the drops *with respect to the initial length* was smaller than after 1 day. This would seem to show that the drops of 0.22 then began to get smaller and the saccharose drops of 0.20 larger. This, then, is a phenomenon which also occurred with the cell-sap, only in a greater degree, so that the osmotic value of cell-sap which was found to be, say, greater than 0.20 *n* after 1 day, was smaller than 0.20 *n* several days later. Barger and Yamakami merely observe that irregularities occur after a longer period.

The following explanation seems to me the most probable one. Owing to the difference in concentration the drops

with stronger concentration at first, as a result of the distilling over of water and of the diffusion of water along the wall of the capillary, become greater, those with the weaker concentration smaller. After a certain time the difference in concentration will be greatly decreased, the overdistilling of water ceases, and the stream of water diminishes [1]). The dissolved molecules and ions of the solution whose osmotic value it is desired to find, will, however, have a different rate of diffusion than the standard substance, boric acid or saccharose, e.g. The more alike the two concentrations become, the more strongly this difference in rate of diffusion may make itself felt. If we have cell-sap and saccharose (low rate of diffusion) in a tube, we can imagine that, when the difference in concentration has become small, the salt molecules and ions of the cell-sap go more quickly through the connecting layer of liquid to the sugar drops, where they increase the concentration, than sugar molecules to the cell-sap drops. If it were found after 24 hours that the cell-sap drops became longer and sugar drops of a particular concentration smaller, then the changes in length of the two sorts of drops may, as a result of the cause mentioned, become, after a still longer time, reversed.

The same phenomenon may of course occur when two chemical substances which have different rates of diffusion are compared with each other. This difference with respect to the rate of diffusion of saccharose is obviously smaller in the case of the above-mentioned substances (glucose, boric acid, urea) than between various standard solutions and the molecules and ions of cell-sap.

[1]) As Yamakami observes, the stream of water to the more strongly concentrated drops will bear along with them molecules of the more weakly concentrated ones; this will, of course, to a certain extent render the attainment of an equilibrium more difficult.

6. *Variability of the Osmotic Value of Cells grown under the same Conditions.*

The variability of the osmotic value is comparatively slight in proportion to the accuracy of the Barger method; that of cells grown in urea (see p. 347) is greater.

An instance of the variability may be given here: the values of 10 cells from water, measured on the same day, were 0.25, 0.25, 0.24, 0.26, 0.26, 0.24, 0.26, 0.25, 0.26, 0.26 $n_{\frac{1}{2}}$ differences, that is, of about 8 %. In 1930—'31 I sometimes mixed the sap of 2—4 cells very quickly on a glass slide, and made 1 or 2 determinations with this; these values are marked with *. If one has enough material at one's disposal, it is advisable to make 2 of such determinations; in this way a better average is obtained than when the average is taken of, say, 2 or 3 separate cells. If one has not enough cells, one must rest content with determining the value of several separate cells. If it is desired to find the difference between the osmotic value in a normal state and at incipient plasmolysis (p. 277) of one and the same cell, or if one wishes to compare the value of a cell in a state of incipient plasmolysis obtained according to Barger's method with the value obtained by another method, (De Vries) (see below), it is also necessary to work with the sap of that cell.

It should further be observed that not all Characeae have such a slight variability in osmotic value. Jost (1929), for instance, states that considerable differences occur in the case of Chara coronata with the various cells of one "wreath of leaves", and even with one "leaf".

7. *Comparison of the Osmotic Value obtained by the Barger Method with that obtained by the De Vries Plasmolytic Method.*

I thought it advisable to compare the osmotic values obtained with the Barger method with the values obtained in another way. The only method which seemed

suitable for this was the plasmolytic method. Nitella cells were put into a solution of saccharose, which just plasmolysed them, this sugar concentration being noted. Cells which had a length of at least several cm., and I invariably used such for my determinations, were found to display in proportion to the accuracy of the Barger method a comparatively slight variability in their values at incipient plasmolysis, just as is the case with the normal values. Only very young cells of a length of several mm. had an incipient plasmolysis-value which was several hundredths higher than with large cells.

I always used saccharose as a plasmolytic. In spite of the fact that it diffuses much more slowly than e.g. KNO_3 (see e.g. De Vries 1877 and Fitting 1917), it is the most suitable, as it does not in practice permeate through the protoplasm in such a comparatively short time as is required for plasmolysis (Beck 1926).

I made the plasmolytic determinations as follows. Two glass strips 30 cm. long and 1½ cm. wide, were stuck on to a glass plate, also 30 cm. long, and 5 cm. wide, by means of shellac, a groove of 1 cm. being left free in the middle. With paraffin any portion of this groove may be shut off as desired. In such a portion a few dried Nitella cells were laid, a sugar solution of known strength being poured over them. Over the groove a glass plate was laid, to prevent evaporation; care must be taken that the liquid does not get between the plate and the glass cover as a result of capillary action, the glass cover must not come into contact with the sugar. In this sugar solution the cells remained half an hour; if they were then still not plasmolysed, the sugar was then poured off and a 0.02 *n* stronger solution was poured on the cells, the latter being first washed twice with the solution which was to be poured on them, so as to remove all trace of the previous concentration. The cells themselves, which, if no longer properly turgescent,

are very delicate, were not touched. The sugar solution is changed until plasmolysis can just be observed. This is done by means of the miscroscope; when plasmolysis is just commencing a few hollows are seen. The cells must have a clean wall; they must not be covered with algae and other impurities. This renders the observation of plasmolysis much more difficult, and frequently even impossible.

As a general rule in plasmolysis experiments cells must be allowed to remain for a considerable time in saccharose; this depends on the object. Fitting (1911, 1915, 1917) takes 2 hours, Höfler 1—2 hours, Ursprung 2 hours, Gratzy—Wardengg (1929) 2 hours (fern prothallia), Beck (1926, 1929, *a*) 30—45 minutes.

With Nitella there is no need to wait so long, since every cell lies open and exposed, and is surrounded on all sides by the plasmolytic. I found that, if they were not plasmolysed after half an hour, this did not occur even if I waited longer.

When the sugar solution which just plasmolyses the cell has been found, the latter is then taken out of the solution, dried quickly, the cell-sap is drawn up by means of a drawn-out capillary, and the value determined according to the Barger method. I first used cane-sugar as a standard solution, later boric acid. To compare these values with one another, either 0.02 *n* must be deducted from the „boric acid values" or 0.02 *n* added to the „saccharose values" (see p. 245). In the following table (13) the first column shows the osmotic values of 10 cells determined according to De Vries. The osmotic value at incipient plasmolysis of the first 5 cells was then determined according to Barger with boric acid as standard solution (second column). After this (third column) are found these 5 values converted into „saccharose values", to permit of a comparison with the values, found directly with saccharose as standard solution of 5 other cells (last 5 figures in the third column). As I

used saccharose as plasmolytic, the values in the first column should therefore be compared with those in the third. The fourth column shows the difference.

TABLE 13

De Vries	Barger		Difference between d. Vr. and B.
	Boric Acid	Sacchar.	
0.29	0.28	0.26	0.03
0.29	0.29	0.27	0.02
0.29	0.31	0.29	0.00
0.30	0.30	0.28	0.02
0.29	0.29	0.27	0.02
0.29	—	0.27	0.02
0.29	—	0.27	0.02
0.26	—	0.23	0.03
0.28	—	0.26	0.02
0.30	—	0.27	0.03

We see that the values found according to De Vries are on an average 0.02 *n* higher than those found by the Barger method. To find an explanation for this fact we must in the first place bear in mind that at the moment when I pricked the cell to let the liquid rise into a tube, there was equilibrium between the sugar solution and the liquid in the vacuole, and that theoretically, therefore, the same value would necessarily be found for both liquids. The phenomenon has therefore nothing to do with possible faults peculiar to the De Vries method of finding the osmotic value in a state of incipient plasmolysis (see p. 232); I therefore tried to find whether there were any faults in the Barger method.

On p. 242 I have already stated that impurities in the cell-sap produce a lowering of the osmotic value. If, then, I had found a lower value in the case of an odd cell with the Barger method than with the De Vries method, this

explanation would be acceptable; in table 13, however, 9 of the 10 cells show the difference in question. As I took the liquid very carefully out of the cells, and impurities would, moreover, have been at once noticeable under the microscope, this possible cause must be ruled out.

I was unable to ascertain the cause of the difference Barger—De Vries [1]); the none the less fairly close agreement gives, however, valuable support to the practicability of the method first named.

In this connection another investigation may be mentioned-that of Maximow and Lominadze (1916, cited by Walter '31 *d*), who compared the osmotic values of press-sap from various plants obtained by the plasmolytic method with those determined by the Barger method. According to Barger the values were up to 3 atmospheres (= about 0.1 *n*) smaller than with the De Vries method. Although we have here to take into consideration that by the plasmolytic method *Oincip* and by the Barger method *Onorm* [2]) values are found and that press-sap and not pure vacuole liquid was worked with, part of this difference will probably be due to the same cause (which I was unable to discover) as with my comparative determinations.

8. *Difference in Osmotic Value between Cells in a State of Turgescence and in a State of incipient Plasmolysis.*

The value at incipient plasmolysis in saccharose was determined according to the De Vries method, the cells were then again put into water, in which in a very short time, in less than one minute, they again became turgescent. The normal value was then determined by the Barger

[1]) The reasons why I prefer Barger to De Vries in my determinations have been dealt with in detail on p. 232.

[2]) *Oincip* is the osmotic value at incipient plasmolysis, *Onorm* in a normal state.

method, partly with saccharose, partly with boric acid as substance for the comparison solutions.

In table 14 the first column contains the values found with the plasmolytic method, i.e. incipient plasmolytic values. In order, however, to compare these with normal values (in a turgescent state) found by Barger's method, the De Vries values must be converted into values such as I should have found by Barger's method if I had made use of it, i.e., by deducting 0.02 *n* (see p. 276 tab. 13); these converted values are found in the second column. In the third column are the values of the same cells after they had again been brought into a turgescent state (determined according to Barger). Where not found directly with saccharose as comparison substance, they have been converted into such values (by deducting 0.02 *n* from the „boric acid values"; see p. 245). In the last column is found the difference between osmotic values at incipient plasmolysis and in a turgescent condition.

TABLE 14

De Vries (plasmol. method)	De Vries — 0.02 n	Barger (values in turg. state)	Difference
0.31	0.29	0.28	0.01
0.29	0.27	0.24	0.03
0.27	0.25	0.22	0.03
0.29	0.27	0.24	0.03
0.29	0.27	0.24	0.03
0.28	0.26	0.23	0.03
0.28	0.26	0.23	0.03

The difference is seen to be, except in the cause of 1 cell, 0.03 *n*. It should be pointed out that this difference is *only* to be found with the Barger method; the method of Ursprung and Blum fails us in this case (see p. 235).

With regard to the possibility that the *Oincip* [1]) found plasmolytically is somewhat too large, see the remarks on p. 233.

9. *Literature relating to the Barger Method.*

As early as 1903 Barger published a preliminary note, in which the principle is very briefly set forth. On the advice of Errera (Brussels), who first thought of it, Barger worked out this method and published, in 1904 (*a*) a detailed article with very numerous examples, which proved it to be excellently suited for the determination, with very considerable accuracy, of the molecular weight of organic substances (the deviation was in these cases generally not more than 5 %). If the concentration of the substance in question, in percentages, and the molecular concentration of the standard substance (boric acid, e.g.), are known, the molecular weight can easily be calculated by this method if it has been determined with what molecular concentration of the standard solution a solution of the substance to be tested gives no change in the length of the drops. With more than 100 substances in various solvents Barger proved the accuracy of his method (for the method itself see p. 238 et seq.). Barger also published this in other periodicals (see p. 238).

Fitting (1917) rejects Barger's method as being very insensitive. For his experiments (determination of isotonic coëfficients) it undoubtedly is so, for my purpose, however, an accuracy of 0.01 *n* is sufficient. Barger was also able to apply his method to determine whether substances associated or not in particular solvents (1904, *b*, 1905), as also whether dissociation occurred (1904, *a*, p. 304). Barger and Ewins determined the molecular weight of epiniphrine

[1]) *Oincip* is the osmotic value at incipient plasmolysis. Ursprung and Höfler, and also Beck, call this value in German Og (Ogrenzplasmolyse).

with it (1906). Loewe (1912) was able to show by means of Barger's method that lipoids dissolved molecularly and not colloidally in organic solvents, Winfield (1912) determined with Barger's method the concentration of blood serum and urine. For the standard solution he took boric acid, as Barger does, with intervals of 0.1 ° C. lowering of the freezing point (= about 0.04 *n*, see tableWalter '31, *d*). Between these he interpolated, which I do not consider permissible (see pp. 269). Yamakami (1920, *b*) used the Barger method for an investigation as to the solubility of caseinogen in alcoholic solutions.

Yamakami (1920, *a*) (see also p. 239) made a detailed and accurate study of the Barger method, which demonstrated that the chief cause of the change in the length of the drops was not distillation of the solvent of the more weakly concentrated drops to the stronger ones, but that, owing to the fact that during the filling of the capillaries these become damp inside, water passes through this layer of liquid from the more weakly concentrated drops to the stronger ones. This water, moreover, will carry dissolved molecules along with it, which will still further promote an enlargement of the stronger drops and a diminution in size of the weaker ones. The experiments which he made on this point to my mind fully prove his statement.

When two drops of different concentration were brought into a capillary in the same way as Barger did, and also into a second capillary, but in such a way that the part of the capillary between the 2 drops did not come into contact with solution, i.e., certainly remained dry (for this method of filling see Yamakami), the change in the length of the drops in the second case was found to proceed 5—6 times more slowly. A second experiment was as follows. If the difference in vapour tension were the only cause of the change in the length of the drops, the extent of the change in volume of 2 drops of different concentration, if allowed

to enter a capillary according to Barger, would necessarily be equal to the difference between their diminution, if each of the two drops were put into a tube apart. In the first case, however, the change is 5—6 times greater! There must therefore be another factor present in the Barger method.

Finally Yamakami says, very truly, that if the changes occurred only as a result of the exchange of vapour tension, the changes would cease when both kinds of drops in a tube had reached the same concentration. If, for instance, a watery urea solution of 0.25 *n* alternating with 0.50 *n* is put into a capillary, then the diminution in size of the drops of 0.25 *n* must cease before their length is half of the initial length, for the concentration would then be 0.50 *n*. The diminution of the drops, however, went on much longer, the length became as little as one-third of the initial length! This can only be understood with the help of Yamakami's explanation. That interpolation, as I have already stated more than once (p. 269), is not desirable, is also clearly shown by Yamakami's experiments.

In his first publication of 1904 Barger pointed out that it takes such a long time before equilibrium is reached; I also noted this during my experiments. Yamakami has to my mind given the correct explanation of this.

If a layer of liquid be assumed on the inside wall of the capillary, Barger's opinion, that this layer immediately collapses into convex drops, which, by evaporating, cause an increase of all the drops to be measured (see pp. 267), is no longer altogether tenable. It seems to me probable that the continuous layer of liquid partly collapses into drops and partly remains continuous, so osmosis can still take place. Both Yamakami's experiments and the phenomenon above described are then explicable.

There has been no lack of attempts to improve the Barger method. Barger and Ewins (1905) constructed a thermo-

stat, which was kept at 70—95 deg. C. with warm water, at which temperature the change in the length of the drops more quickly occurs. Barger also describes this apparatus in 1915. As a temperature of 90 deg. on cell-sap seems to me somewhat risky, I did not make use of this thermostat.

Rast (1921) employed much longer capillaries and took the (seven) drops much longer than Barger did, about 1.5 cm. long. He states that a difference in concentration of 0.0008 *n* could be determined in this way. The drops were measured with a finely divided glass scale, on which the tubes were stuck. This greater accuracy is naturally an advantage, but the measurement seems to me likely to take longer, besides which much more liquid is required; this latter was in itself a reason why I could not make use of this method. Moreover so high a degree of accuracy would be of no use to me (see p. 234). Rast (1923, 1924 and also as already stated in 1921) afterwards simplified the method very greatly by alowing only 1 drop of the standard solution and 1 drop of the test liquid to enter the capillary. (The manner in which filling was carried out cannot be described in detail here). Both drops were 4 cm. long, and show a much greater change in length than is the case with smaller drops. This simplified method is less accurate than Rast's first method. It was of equally little use to me, since it demands far too much liquid. The Rast method is included in Emich's textbook of microchemistry (1926, p. 112). Pringsheim and Lassmann (1922) applied it in their determinations of the molecular weight of inuline and glycogen.

Hrynakowski and Rychter (1925, critical review in Chem. Zentr.bl. 1926) improved the simplified Rast method by keeping the temperature constant at 0.2 to 0.3 deg. C., with the aid of electrical heating. In this way differences could be shown of 0.005 *n*. The reasons why I did not make use of this method are the same as those mentioned by Rast.

Hrynakowski and Rychter determined, with their method, the osmotic value of blood serum, and obtained values which agreed well with the values found kryoscopically by others.

Hrynakowski (1924, Chem. Zentr.bl. '25) considers the „distillation isothermique" from the theoretical side, but with his thermodynamic considerations does not take any account whatever of the fact that the change in the size of the drops is brought about to a great extent by diffusion! (Yamakami). I do not consider this article very clear in connection with what Hrynakowski and Rychter say in their following publication (1925): with the Rast method an equilibrium is reached in as little as 2 days; incidentally they further remark that when they took care that the wall remained dry between the two drops in the capillary, a change in length none the less took place, which indeed Yamakami, whose name they barely mention in 1924, also found. Do the remarks expressed in the publication refer to this way of filling? I was unable to make out whether this was so or not.

Let us now see what applications of the Barger method have been made in the domain of botany. The first to determine the concentration of plant sap with it was Miss Halket (1913). She determined the osmotic value of press-sap from Salicornia, Cotyledon, and a few other succulent plants. I have discussed the disadvantages and the literature of working with press-sap, in comparison with vacuole liquid such as is to be obtained from Nitella, on p. 237; I will not revert to these here. With the restrictions mentioned on p. 237 it will certainly be possible to determine the osmotic value of press-sap from plants (organs) by the Barger method, *provided this sap is obtained in the most approved manner* (see e.g. Walter '28, '31, *c*, '31, *d*) and if it is taken into account that the osmotic value of cell-liquid mixed with liquid from the protoplasm, vessels and cell-walls is being determined.

Miss Halket, who professes to be able to determine the osmotic value of a single leaf, says, however, on p. 175 „The juice of the organ can be squeezed out by pressure of the fingers and the drops obtained can be used directly to fill the tubes"! Such a rough way of obtaining the liquid can never give a true result. In the first place it is probable that only juice out of the outer part of the organ is obtained. And Ursprung and Blum (see also Beck 1931) have shown in their various publications that the osmotic value of all kinds of cells is far from being alike. There are also other conditions, discussed on p. 237, which are not fulfilled by Miss Halket[1]). She furthermore uses NaCl as substance for the standard solutions, a substance which should preferably *not* be used, as it is dissociated (Barger). Miss Halket worked with intervals of 0.01 *n*; according to her published tables she actually does get good differences, but she only gives one determination of each plant, so that it is impossible to gather whether this is really *the* value of the plant (or leaf), as checking determinations are lacking. Nothing is said as to impurity of the liquid, which of course exists, and this may produce differences of several hundreds *n*. (see p. 242).

Ruhland (1915, *a*) uses Barger's method for the determination of the concentration of secretion liquid from Statice, and praises this method very highly, especially since so little liquid is required. As a standard solution Ruhland takes NaCl solutions rising in concentration by 0.25 %, so finding with what per cent concentration of NaCl the secretion is isotonic. It is, however, undesirable, as Halket's determinations, to use this substance, even if the secretion also contains to a great extent NaCl (with which Ruhland

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[1]) Miss Halket's determinations were made in 1913, i.e. before Walter, Ursprung, etc. published their investigations. The same applies to Ruhland, who discussed Miss Halket's publication in 1913.

was concerned). There are, however, also other substances in it (Ruhland pp. 438, 446).

Maximow and Lominadze (1916), cited by Walter (1931, *b*, p. 325 and especially '31, *d*) also made determinations with the sap of plants according to Barger's method (see in details p. 277). Daumann (1930) determined by Barger's method the nectar secreted by the stigma hairs of Araceae. He took capillaries of a thickness of 0.25 mm. (which I also used), took saccharose as standard solution, with intervals of 0.05 *n*, larger, that is, than I used. A control such as I was able to apply with the plasmolytic method of De Vries was not made use of by Daumann; his determinations, however, seem to me to be accurate.

The present writer (Wildervanck 1931) succeeded in determining the osmotic value of vacuole liquid from one cell of Nitella translucens by Barger's method. In this provisional communication part of the determinations is briefly described which are here communicated in detail.

Ursprung and Blum (1930) published a method which bears much resemblance to that of Barger (see also p. 236). They originally instituted their method for a very considerable quantity of liquid of which the osmotic value is to be determined; they afterwards give a modified method, with which less liquid is required, and finally alter this, too, so that still less liquid is necessary. I will here briefly describe this method only. A saccharose solution of known strength is put into a dish, over this a glass plate is laid, on which a number of glass capillaries, 6 mm. long and 3 mm. thick are stuck. One of these is partly filled with the press sap, into the others solutions of constantly rising concentration are put, one of which solutions has the same strength as the one on the bottom of the dish. This dish with tubes is then hermetically closed. The distance from the meniscus of the liquids in the tubes to the upper end of these is then measured microscopically with an eye piece micrometer. This

is repeated after a certain time, when the distances are found to have changed, owing to the fact that the vapour tensions above the various liquids are different. From this, as is also the case with the Barger method, the strength of the test solution can be deduced. In the tube in which is the same solution as on the bottom of the dish, the distance should theoretically be the same; this distance is found, however, to be greater, a deviation which has to be taken into account when determining the strength of the cell-sap, which naturally renders the method less accurate. How great the degree of accuracy is, the investigators do not say; it is, however, smaller than with their original method, with which the accuracy is 0.02 *n*. The method is therefore less exact than Barger's, it is more troublesome and requires more liquid, so that it is inferior to that of Barger. Remarkably enough, Ursprung and Blum do not discuss the Barger method at all, they merely mention his name.

Walter (1931, *b*) also refers in his criticism of the method of Ursprung and Blum to the greater simplicity and accuracy of Barger's method. With the Barger method a smaller distance between the drops, exclusion of errors resulting from the different consistency of the glass and from inequality of the diameters of the capillaries, and consequently different meniscus curves; a source of error with the method of U. and B. is further the unequal distances from the meniscus to the openings of the various capillaries.

C. ANNUAL PERIODICITY OF THE OSMOTIC VALUE OF THE CELL-SAP WHEN THE CELLS ARE GROWN IN WATER. INFLUENCE OF THE DEGREE OF ACIDITY, TEMPERATURE, AND LIGHT.

The cells of Nitella translucens were found to have a higher osmotic value in winter than in summer, which might be due to anatonosis. Factors which might cause this anatonosis

are the temperature and the intensity of the light, a further possibility being, perhaps, a changed Ph. Before giving the results of my investigations, I think it desirable to give a summary of what is known on this point in the literature; I should like to begin this summary with a few remarks on the constitution of the vacuole sap of Nitella.

I. Literature.

1. *Constitution and Acidity of the Cell-sap.*

I was unable to find any analyses of Nitella translucens in the literature; I found, however, analyses of Nitella clavata, the constitution of which will be approximately the same as that of Nitella translucens. In the cell-sap it was possible to demonstrate the ions K, SO_4, Ca, Mg, PO_4, Cl, and Na (M. M. Brooks 1922, Hoagland and Davis '23, Irwin '23, *b*); Hoagland and Davis concluded from the conductivity that practically all the substances must be dissociated. R. Collander (1930) tested the cell-sap of Chara ceratophylla, a species found in brackish water, and found the same ions; the sap contains about 1.5 % salts and at the most 0.3 % organic constituents, including less than 0.1 % albumen. Of Valonia, a large-celled Alga belonging to the Siphonocladieae Osterhout also observes (1923) that there occur very few organic substances in it. Unfortunately none of the investigators named made any sugar tests; sugars will certainly not be found in strong concentration in Chara ceratoph., if indeed they are present at all, in view of the low percentage of organic substances (0.3 %). In the cellsap of Nitella translucens no fermentable carbohydrates were found to occur either in summer or in winter, as my investigation showed (see p. 328), but the chloroplasts contained a great deal of starch, which agrees with what Oltmanns (1923, part III, p. 199) states: „Charales readily produce starch, as can be demonstrated without difficulty".

The osmotic value in Nitella will be caused, not only by the ions mentioned, but also by organic acids, of which De Vries has shown the importance in connection with the osmotic pressure (1883, '97), this being confirmed by Pfeffer (1897, p. 121) and Ruhland (1915, *b*).

The osmotic value at incipient plasmolysis of Chara ceratophylla is, according to Collander (1930), 0.17 *n*. That of Nitella translucens I found to be approx. 0.27 *n* (summer); I further determined the value (in a turgescent state) of a smaller species of Nitella, which was about 0.28 *n*.

With regard to the *Ph of the cell-sap* of Nitella clavata, this was found by Hoagland and Davis (1923) to be approx. 5.2, which value remained constant when the Ph of the external liquid was changed from 5.0 to 9.0! The same thing was found by Crozier (1919) for Valonia (value approx. 5.9). Taylor and Whitaker (1928) found the Ph of Nitella spec. to be about 6.0. For fuller details, also of other Algae and for the methods, see Small's monograph (1929). For the influence of the Ph of the environment on the osmotic value, I may also refer to this work. As I cultivated the Algae in main-water, I consider it unnecessary to deal with the influence of the Ph.

2. *Annual Periodicity of the Osmotic Value of Plant Cells.*

I was unable to find anything in the literature about the annual periodicity of the osmotic value in the case of Algae. Determinations have, however, been made with higher land-plants; as with these all kinds of factors come into play which are not present in the case of water Algae, such as wind, moisture of air and soil, rainfall, a comparison of the periodic phenomena of Nitella with these plants is of little use. I will therefore confine myself to a few remarks and references to the literature. Most writers find a higher value in the winter than in the summer (Lidforss 1907, Kny 1909, Winkler 1913, Meier 1915, Ursprung and Blum

1916, *a*, Gratzy Wardengg 1929, Beck 1929, *b*). The difference sometimes only amounts to a few hundredths *n*, with other plants, on the other hand, the winter value is twice or even three times as much as the summer value.

Several investigators find with their test plants no connection between the time of year and the osmotic pressure (Lewis and Tuttle 1920, Lambrecht 1929). Walter, (1931, *d*) made interesting observations with regard to the connection between periodic changes of the osmotic pressure in different climates.

3. *Influence of Light on the Osmotic Value.*

De Vries (1884) was again one of the first to study the influence of light and darkness on the osmotic pressure. Etiolated stalks, according to him, have a lower osmotic value than under normal conditions; Copeland (1896) confirmed this partially, with the reservation, however, that this was only the case when they grew more rapidly than under normal circumstances.

Dixon (1914) investigated leaf-cells for their osmotic value, and found these to be greater when the cells were exposed to light than in the dark.

Buchheim (1914) is one of the few who studied *Algae*; the turgor became greater, in the case of Cylindrocystis and Spirogyra, under strong illumination. Ursprung and Blum's determinations (1916, *b*, p. 130), which showed leaves in the shade to have a lower osmotic value than leaves in the sun (difference approx. 0.04 *n*), are of little value to me, as difference in temperature is also a factor of importance here. J. Meier (1915) reports the same of alpine plants. Blagowestschenski (1926, '28) repeated, as it were, Ursprung and Blum's determinations experimentally; with leaves protected from the sunlight he found the osmotic value to be lower than with exposed leaves. Bächer (1920) carried out more detailed determi-

nations, he determined the effect of light at various temperatures in the case of Elodea canad., and found that at 2500 candle-power the *Oincip* [1]) became about 0.05 *n* greater than in the dark; at higher temperatures this difference becomes still greater. Exposure to sunlight causes a slighter difference, at 18 deg. C. none at all; at 20 deg. his values were 0.42 *n* (sun) and 0.39 *n* (dark), at 24 deg. 0.42 *n* and 0.40 respectively. He obtained similar results with Aspidistra.

Lambrecht (1929) found that light increased the osmotic value in the case of mosses, as also with full-grown Phanerogamae; he found no difference in the case of protonema of mosses; with cotyledons light, he states, causes a slight diminution of the osmotic value. Miss Gratzy—Wardengg (1929) also comes to this latter conclusion in the case of fern prothallia.

With one or two exceptions, thus, higher values are found in light than in the dark. Of especial interest to me were the results of Buchheim.

4. *Influence of the Temperature on the Osmotic Value.*

It is again the same investigators who studied the effect of light that we find here.

Copeland (1896) found a high osmotic value with a temperature of 37 deg., and also with 1 deg.; between these two (18 deg.) the value was lower. Pantanelli (1904 p. 328) found something of the same kind with Aspergillus. Buchheim (1914) made a study of Cylindrocystis and found a higher osmotic value with a low temperature: at 15—20 deg. 7.75 % saccharose, at 8—10 deg. 8.75 %, at 0 deg. 10 %. J. Meier (1915) found the same thing with

[1]) By *Oincip* I invariably mean the osmotic value at incipient plasmolysis, Ursprung and Blum's Og (grenzplasmolyse).

a number of leaves; for instance with Taraxacum 0.35 *n* and 0.50 *n* at 15 and —1 deg. respectively, with Genista tinct. 0.50 *n* and 0.60 *n* at the same temperatures, with Primula Auricula 0.35 *n* and 0.60 *n* respectively. Ursprung and Blum (1916, *b*, p. 125) publish investigations on leaves; they usually find a somewhat higher value in the cold than with high temperatures. The light factor is, however, not eliminated with these experiments (see p. 289). Bächer (1920) investigated a several higher plants and Cladophora, his experiments are more exact than those of most other writers; he determined the osmotic value at temperature intervals of 5 deg. With Cladophora insignis he found, at 0 deg. C., the values 0.90 *n*, at 5 deg. 0.88 *n*, at 10 deg. 0.86 *n*, at 15 deg. 0.83 *n*, at 20 deg. 0.80 *n*, at 25 deg. 0.77 *n*, and at 30 deg. 0.75 *n*; these increases began to occur within 1 day. When the temperature was raised he found with the other plants also a fall in the osmotic value. In some cases he again found a rise above 30 deg. C., something of the same kind, that is as Copeland and Pantanelli describe.

According to Elfr. Gratzy—Wardengg (1929) fern prothallia react even at a reduction of temperature of a few degrees only, with a rise in the osmotic value. Above 25 deg. the value again rises. Cultures kept during the winter in a warm laboratory remain at a low value, which rises to the winter value after so short a time as 7 hours when the plants are taken out into the cold outside air. I regard this observation as of importance in connection with my experiments (see p. 298), although Gratzy—Wardengg says nothing as to the constancy of the temperature, and her description gives one the impression that the influence of other factors is not entirely out of the question. The qualitative results are certainly worth noting. For the influence of the temperature see further Stiles (1924, p. 118).

To sum up we may say that all investigators *find a lower osmotic value at higher temperatures.*

II. My Investigations.

1. *Annual Periodicity of the Osmotic Value.*

The osmotic value (in mol p. liter, *n*) was determined from 1927 to 1931, the values found being shown in tables 15*a* and 15*b*.

In both tables is found under „date" the day of the month in which the determinations were made, the name of the month being in the first column. Values marked with an * were obtained by mixing the cell-sap of 3 or 4 cells (cf. p. 273), the winter values, which are higher than those found in summer, being in heavy type. The dates of 1927 and 1928 in tab. 15*a* are indicated by a dash only, as I had not noted the dates of these values exactly at the time.

TABLE 15*a*

Month	1927			1928		
	Date	Osmot. values in *n*.	Average	Date	Osmot. values in *n*.	Average
April ...	—	0.26, 0.25, 0.27, 0.26, 0.25	0.26			
May ...	—	0.26	0.26			
July....	—	0.27, 0.27, 0.26, 0.24	0.26			
Aug....				—	0.24, 0.24, 0.25, 0.24, 0.27	0.25
Sept....				—	0.25, 0.26, 0.25	0.25

Osmotic value in '27 and '28

We see from tables 15*a* and 15*b* that the value in summer is invariably about 0.26 *n*, which corresponds to approximately 7 atmospheres (see table Ursprung and Blum 1916, *c*, and Walter '31, *d*). In the latter part of January 1929, the value was 0.39 *n*, afterwards gradually falling to the

TABLE 15*b*

Month	1929			1930			1931		
	Date	Osmot. values in *n*.	Average	Date	Osmot. values in *n*.	Average	Date	Osmot. values in *n*.	Average
Jan.				1	0.60*	0.60	5	0.31*	0.31
	20	0.38, 0.40	0.39	22	0.51*, 0.50	0.51	23	0.30*, 0.30	0.30
Febr. ..				6	0.31*	0.31			
				8	0.27*, 0.27*	0.27			
				10	0.29	0.29			
March .	—	0.30, 0.33	0.32	8	0.33, 0,32	0.33			
Apr. ...	20	0.29, 0.30	0.30						
June....	8	0.32, 0.31	0.32				5	0.29*, 0.29*	0.29
	20	0.30, 0.28	0.29						
July....	7	0.30, 0.29, 0.29	0.29	5	0.30	0.30	13	0.30*	0.30
	9	0.31, 0.32	0.32				16	0.31	0.31
							24	0.28, 0.29	0.29
Aug.	3	0.28, 0.30, 0.28	0.29	16	0.26, 0.28	0.27	4	0.25, 0.26	0.26
	24	0.30, 0.30	0.30	20	0.26*	0.26	19	0.26*, 0.27	0.26
	27	0.28, 0.27, 0.27	0.27						
Sept....	7	0.27, 0.27,		8	0.28	0.28	1	0.27, 0.27	0.27
		0.27, 0.28	0.27	11	0.25, 0.26, 0.26	0.26			
	17	0.26, 0.27,		18	0.25, 0.25	0.25			
		0.27, 0.25	0.26						
	30	0.28, 0.28, 0.26	0.27						
Oct. ...	5	0.26, 0.28		1	0.29	0.29			
		0.25, 0.25	0.26	17	0.26, 0.26	0.26			
	19	0.26, 0.27, 0.25	0.26	20	> 0.30	>0.30			
	23	0.27	0.27	23	0.30, 0.32	0.31			
				26	0.34	0.34			
Nov. ...	3	> 0.34, > 0.34	>0.34	1	0.33*	0.33			
	8	> 0.38, > 0.38	>0.38	3	0.32	0.32			
	9	> 0.46, > 0.46	>0.46	5	0.33*, 0.33	0.33			
	11	0.50, 0.51, 0.49	0.50	10	0.31, 0.34	0.33			
	12	0.48, 0.49	0.49	20	0.33	0.33			
	27	0.61, 0.60	0.61						
	29	0.62	0.62						
Dec. ...	4	0.61	0.61	2	0.32	0.32			
	14	0.62, 0.60	0.61	5	0.33, 0.33	0.33			
	17	0.62, 0.60	0.61						
	22	0.59*, 0.58	0.59						

Osmotic value in '29, '30 and '31

summer value of 0.26 *n*. At the end of October the rise recommences, at the beginning of December the maximum of about 0.60 *n* is reached, i.e. approx. 17.5 atmospheres. In January 1930 the value falls again, this fall coming to a standstill in August, the value being then 0.26 *n*. At the end of October it again rises to a maximum of from 0.33 to 0.34 *n*, much lower than in the winter of '29—'30, and also less than in January 1929. In the summer of 1931 the osmotic value again reaches 0.26 *n*. In figs. 3 and 4, p. 320, 321 the changes in the value in '29 are shown by means of a graph. *Summarizing the data, we see that in summer the osmotic value is constantly 0.26—0.27 n, in winter the value is higher, but not to the same extent every year*. The obvious supposition was that the temperature and the light were the factors which caused these variations in osmotic value. On this point I have also carried out a number of researches.

2. *Effect of Light on the Osmotic Value.*

On December 18th 1929, Nitella cells were taken from the Botanical Garden, where the temperature had for a long time been low, and put partly in the dark at 25 deg. C. and partly in the light at 25 deg. C. The source of light used was a 100 candle-power Philips Argenta lamp, suspended in the upper part of a thermostat, which was divided into two parts by a horizontal partition. In the lower half it was dark, in the upper one light. In this upper part the glass dishes with Algae were placed at a distance of 20 cm. from the lamp, whilst between the dishes and the lamp a glass plate was placed to prevent too great heating. In light the cells do not keep good so long as in the dark. Table 16 shows the result. Values marked * were again obtained with cell-sap from several cells which was mixed. On Dec. 22nd determinations were begun, after the cells had been for 4 days at 25 deg. in the dark and in the light respectively.

TABLE 16

Date	Dark, 25° C.	Light, 25° C.	Difference
22–XII–'29	0.61, 0.60	0.62, 0.62	0.01
27–XII–'29	0.60*, 0.60*	0.63*	0.03
1– I–'30	0.56, 0.57	0.61, 0.61, 0.60	0.04
22– I–'30	0.50*, 0.51	0.53*, 0.54	0.03
10– II–'30	0.33*, 0.34	0.36*, 0.36	0.02

These determinations were repeated in the summer of 1930, when the value was found to be 0.26 *n*. After the cells had remained at 22 deg. for 10 days, I began the determinations. See table 17.

TABLE 17

Date	Dark, 22°	Light, 22°
24– VII, '30	0.24, 0.26	0.26*
28– VII, '30	0.25*, 0.24	0.26*, 0.25
8–VIII, '30	0.27*, 0.26	0.26*, 0.26

We note that *with the winter cells (table 16) the value of the cells in the dark is several hundredths normal smaller than in the light,* which agrees with the results of the vast majority of the investigators mentioned in the summary of the literature (p. 289); as this manifested itself in all the determinations this difference may be regarded as an actual one.

In the case of the summer cells (tab. 17) we still see no difference, after 15 days, between lighted and unlighted cells. I should point out that *the difference found* (in the case of winter cells) *cannot account for the annual periodical changes* (in winter high, in summer low), for, if it were the light factor which caused the change, we should expect to find high values in summer (long days, strong intensity of light) and low ones in winter, whereas precisely the reverse is the case.

An important fact which may be deduced from tab. 16

is *that the periodic fall which begins as early as January, also occurs at 25 deg. C. and both in the light and in the dark,* i.e., not only at room temperature and outside.

3. *Influence of the Temperature on the Osmotic Value.*

Algae were put into the dark at 9 deg. C. (in a refrigerator) on November 13th, 1929, and another lot into a thermostat at 25 deg. C.; the temperature fluctuated at the most 1 deg. On that date the cells had a value of about 0.50 *n.* From Nov. 27th., when the cells could be expected to have adapted themselves completely to the temperature, the osmotic value was determined. The values marked * were obtained by mixing cell-sap from 4 or 5 cells. It should further be pointed out that at 25 deg. the cells did not keep good so long as at a lower temperature.

TABLE 18

Date	9°, dark	25°, dark	Difference
27– XI–'29	0.61, 0.60	0.54, 0.55	0.06
29– XI–'29	0.62, 0.60	0.55, 0.56, 0.55	0.06
4–XII–'29	0.61*, 0.62	0.56	0.05
14–XII–'29	0.62, 0.60, 0.60	0.54, 0.55*, 0.55	0.06

The same determination was again made in the summer of 1930, at 22 deg. and 1 deg. C., the value being then about 0.26 *n.* After the Nitella cells had been at this temperature for 10 days, a commencement was made with the determinations (table 19).

TABLE 19

Date	1°, dark	22°, dark	Difference
24– VII–'30....	0.28	0.24, 0.26	0.03
28– VII–'30....	0.28*, 0.27	0.25*, 0.24	0.03
8–VIII–'30....	0.28*, 0.28	0.27*, 0.26	0.01

In January, 1931, a few more comparative determinations were made between cells which had been the whole winter in the heated laboratory room (temperature about 18 deg. C.) and Algae from the Botanical Garden, where it was much colder, and there was even occasionally frost (tab. 20). Unlike the two previous experiments, tab. 18, 19 both groups of Algae were cultivated in the light.

TABLE 20

Date	Laborat. (hot) Light	Botan. Garden (cold) Light	Difference
5–I–'31	0.31*, 0.30	0.34*, 0.33	0.03
7–I–'31	0.29*, 0.30	0.34, 0.32, 0.33	0.04

If we examine tables 18, 19 and 20, we find that winter cells have a value of about 0.06 *n* *in the dark* at 25 deg. lower than at 9 deg. (tab. 18), summer cells a value about 0.02 *n* lower (tab. 19). *In the light,* with cells which are already „on the way" to the summer value, the value in the cold is 0.03 to 0.04 *n* higher than in the warmth (tab. 20). The phenomenon high temperature — small osmotic value and low temperature — high osmotic value agrees with what was found by most other investigators (see p. 290).

The fact that a low temperature has the effect of increasing the osmotic value cannot, however, account for the high winter value of January, 1929 (0.39 *n*), and still less for the winter values of '29—'30 (0.60 *n*). The difference between the summer value 1930 and the winter value '30—'31, which is about 0.07 *n*, is, moreover, greater than could be brought about by a difference in temperature. It must, however, be further borne in mind that the values shown in tables 15*a* and 15*b* all refer to Algae cultivated in the laboratory, as a result of which the change of temperature from summer to winter was practically eliminated, so that the periodical changes in the laboratory are practically inde-

pendent of the factors light and temperature, whilst in the open air these are far from sufficing to account for the periodic difference.

The case with Nitella is thus different from that of the fern prothallia of Miss Gratzy—Wardengg, (see p. 291), which, when they were kept in the warm laboratory, retained their low values.

Summarizing we see that *in light and in dark the osmotic value is higher at a low temperature than at a high temperature, this rather small difference however cannot account for the difference between the high winter values and the low summer values.*

4. *Anatanosis as Cause of the Periodic Change of the Osmotic Value.*

We have noted that the periodic changes which occur in natural conditions also manifest themselves in the laboratory, where the temperature in winter is not lower than in summer, that even with a constant temperature of 25 deg., and both in the light and in the dark (tab. 16), the value falls at the end of January. It is further seen that cells cultivated in glucose and saccharose also show this periodicity (tab. 21, 22, 23, and 24). For graphs see figs. 3 and 4, p. 320, 321. Moreover, the value is found to be different in different winters, in the summer it is always about 0.26 *n*.

The meaning of this anatonosis for the plant may be, as also suggested by Walter (1926) an adaptation to be better able to resist the cold. Senn (1922, cited by Ursprung (1925) supposes (in the case of field plants) that the sugar formed during assimilation is transformed to a smaller extent into starch in winter than in summer, but according to Ursprung (1925) cells free from chlorophyll also show this increase!

Moreover Nitella is found to contain no sugar in the cell-sap either in summer or in winter, unless grown in fairly

strong sugar solutions. It is true that the chloroplasts contain a great deal of starch (see p. 325); it is possible that more osmotically active substances (acids) are formed from this in winter than in summer. Whatever the source of these substances may be, it is certain *that the increase in the winter is to be ascribed to anatonosis of one kind or another. As the cause of this anatonosis unknown autonomous factors must be assumed.*

D. OSMOTIC ADAPTATION OF NITELLA GROWN IN SUGAR SOLUTIONS.

In this chapter cultivation experiments of Nitella in saccharose and glucose solutions of 0.10 and 0.20 *n* will be discussed; the determinations of the osmotic values were again made according to the Barger method, whilst the micro-method of Lutsenburg Maas—Van Iterson was employed for the determination of the sugar in the cell-liquid. The possible causes of the increase of value found: permeation, and anatonosis are discussed in detail. In the discussion of the anatonosis special attention is paid to starch as a source of osmotically active substances. Finally I traced the influence of light and temperature on the increase of the osmotic value of the Nitella.

I. Literature.

There is not entire agreement as to the existence or non-existence of permeability of the protoplasm to sugar. Many deny it altogether, others assume a (slight) permeability. Unfortunately the same thing is not always understood in the literature by the term „permeability". It is necessary to distinguish between:

1*a*. *endosmosis* of substances, in casu sugar, *into the protoplasm* (demonstrable by the formation of starch) and

1*b*. *endosmosis* of the substances in question through the innermost layer of protoplasm, the tonoplast, *into the vacuole.*

In addition there is:

2*a*. *exosmosis*, outflow of substances *out of the protoplasm*. This may play a part in the case of sugars, owing to the fact that starch may be decomposed into sugars, which then pass out into the cultivation solution or water.

2*b*. *exosmosis out of the vacuole* of the cells.

Many investigators confound these four processes; in many cases, indeed, it is impossible to determine which we have to deal with. In the case of endosmosis it will frequently be impossible to say with certainty whether it is 1*a* or 1*b*, this difficulty being still more greatly increased when we endeavour to distinguish between the two kinds of exosmosis. Some, however, when exosmosis has been demonstrated, say that „permeability" exists, whilst very many understand by this endosmosis exclusively. Increased endosmosis and exosmosis need not by any means always go together; Janse (1888) found, for instance, with Chaetomorpha and Spirogyra, that KNO_3 and saccharose permeated inwards, but afterwards, in water, did not flow out again; there was endosmosis but no exosmosis. See in this connection also the remark as to Schimper's investigation on p. 303. Zycha (1928 p. 502) also calls attention to the confusion between the ideas of exosmosis and endosmosis. He formulates clearly „by permeability I understand the permeability of the protoplasm to a substance from outside into the interior of the cell". Höfler (1931) refers to the necessity of distinguishing between endosmosis into the protoplasm and into the vacuole. Also Weevers (1931) distinguishes sharply between endosmosis of sugar into the vacuole (which is small) and into the protoplasm (formation of starch).

In the summary of the literature I will discuss the investigations on exosmosis of sugars and those on endosmosis as far as possible separately. Exosmosis, which is of less import-

ance to me, is dealt with under *a*; it should be pointed out that it is usually impossible to say whether we have to deal with exosmosis out of the vacuole or only out of the protoplasm. Under *b* is discussed the literature practically exclusively relating to endosmosis into the vacuole; for practical reasons I discuss endosmosis into the protoplasm and the formation of starch from sugar under *c* with the anatonosis.

1. *Permeability of the Protoplasm to Sugars.*

a. Exosmosis.

The experiments made on this point largely refer to reserve organs of plants; in the case of many objects it is possible to demonstrate sugar chemically in the water into which these organs are put; exosmosis therefore occurs, either out of the vacuole or out of the protoplasm. We cannot prophecy as to the possibility or impossibility of exosmosis of sugar from Nitella on the strength of the observations in the literature; as I made no determinations on this point, I do not think it necessary to discuss the investigations of others in detail.

b. Endosmosis.

A fairly large number of investigators put cells, either Algae or leaves, into plasmolyzing sugar solutions, and conclude from the decrease of the plasmolysis after a longer or shorter period that there is permeation of these sugars, a method which is also employed by many for permeability experiments of salts. An odd writer discusses, in connection with experiments of this kind, the possibility of anatonosis; for the sake of clarity I will discuss the determinations which relate more directly to anatonosis separately.

Janse (1887) is one of the first to make communications as to the decrease of plasmolysis in plasmolyzing sugar solutions. Chaetomorpha area was plasmolyzed in *saccharose*, and after 2 hours the plasmolysis had disappeared; although

the possibility of anatonosis is just mentioned, he concludes without any further proof that permeation has occurred. Algae were further plasmolyzed in solutions of 0.16—0.40 *n glucose* (Janse '88), after nine or ten days the cells being still alive and the plasmolysis having completely disappeared.

To keep first to the experiments on Algae, the investigations of Klebs (1886—'88) may next be mentioned: Zygnema, plasmolyzed in *saccharose* of 16—20 %, showed no decrease of the plasmolysis after several weeks; Spirogyra and Vaucheria also remained plasmolysed.

An investigation carried out by Iljin (1928 p. 594) may also be mentioned here: Valonia utricularis (a sea Alga) was cultivated in sea-water, diluted or not. When a varying amount of phosphoric acid was added and further *glucose* 0.1 and 0.2 *n*, it was found chemically that after 24 hours there was more sugar present in the cells than they previously contained; the more acid the reaction of the environment, the more sugar could be shown chemically in the Valonia. It is not impossible that in this case the permeability to glucose is increased by the acid though it is not allowed to exclude the possibility of anatonosis (W.). I will remark en passant that Iljin's figures mean little as absolute values, as in the first place nothing is said as to the number of cells tested, and secondly he sometimes found a totally different content of sugar (sometimes 1.02 %, at others 0.30 %) in pure sea-water with the same Ph; I do not understand this.

Finally Algae were used by Lepeschkin (1908), who states that he finds no permeability to various sugars with Spirogyra, and Lapicque (1921), who cultivated Ectocarpus, a sea Alga, in sea-water in which 0.2—0.6 *n saccharose* had been dissolved. Plasmolysis which decreased partially after 3—4 hours, and entirely after 24 hours; the cells even swelled greatly. He does not deduce permeability from this, but expresses himself somewhat vaguely as to the cause: „il faut admettre une accommodation active de la

cellule, un travail du protoplasme s'exerçant contre la pression osmotique, qui est, non pas le facteur agissant, mais la résistance". He is obviously here thinking of anatonosis, but does not enter further into the matter.

Coming now to the publications on the permeability in the case of *higher plants*, I will begin by mentioning two other writers, who showed the presence of *glucose chemically* (with Fehling's reagent) in leaves which were brought on sugar solutions: Schimper (1885) finds, in leaves of Impatiens parviflora, freed from sugar in the dark, a strongly positive reaction in the leaf-nerves, but much less in the mesophyll, after they had lain for 4½ hours (in the dark?) on 3 % *glucose*; Pfeffer (1886) finds the same with seedlings of Allium in air free from CO_2 grown on *glucose*. Both Schimper and Pfeffer observe that the glucose was not given off into the water; the latter writer therefore states on this point that the phenomenon „von besonderer Lebenstätigkeit abhängt". This shows once again that endosmosis (if, at any rate, it is an endosmosis of the glucose itself (see 5 lines lower down) and exosmosis do not necessarily go together.

Schimper supposes that the glucose which has penetrated into the cells is still further decomposed, so that it may be conveyed in the plant. It is then, according to him, only partially restransformed into glucose in the cells. Moreover, he does not say positively that the glucose *as such* penetrates into the cells from the outside, but leaves the possibility open, that it is first changed in the protoplasm and is then built up again into glucose in the cells.

Let us see what other writers find, also with higher plants. Decrease of plasmolysis in *saccharose* is found by Wieler (1887) in the case of seedlings of Vicia faba. Conclusion: permeability. A strong formation of starch would prove this still more. This latter, of course, only proves a penetration into the protoplasm, not through the tonoplast into the

vacuole (cf. p. 300). Lundegaardh (1911—'12) concludes from the decrease of plasmolysis of the cells of roottips of Vicia Faba that there is a very low permeability to *saccharose.*

Overton (1899) concludes that there is no diosmosis of sugars, as does also Prát (1922) *(saccharose).* Beck (1926) finds no decrease of plasmolysis or increase of the value at incipient plasmolysis after 20—60 minutes.

Tröndle (1910) on the other hand finds that *glucose* has been „taken up" into cells of leaves of Tilia, i.e. deplasmolysis; also after 2 days in 1.2 *n saccharose;* Ruhland (1912) also states that he observed, in the case of Beta leaves after 24 hours plasmolysis in solutions which were not too much stronger than the osmotic values of the cells, a slight deplasmolysis in *saccharose,* and to a greater extent in *glucose* and *fructose.*

A favourite object is, of course, also Tradescantia discolor (Rhoeo). Fitting (1917), after 2 hours plasmolysis finds a very slight decrease in *saccharose,* pointing to permeability, which deplasmolysis, however, „auch in anderer Weise gedeutet werden könnte."

Höfler (1918, *a*), by means of the plasmometric method finds an increase of value of 0.004—0.009 *n* in 0.30 *saccharose* after 24 hours, „infolge einer ausserordentlich kleiner Permeabilität für Saccharose". In a later publication (1926) Höfler traces the decrease of the plasmolysis of cells of Gentiana, Majanthemum, and other plants in *saccharose.* After 24 hours he sees a slow decrease; by means of his plasmometric method he calculated that 1/650—1/700 of the outside concentration was taken up per hour. In *glucose* the plasmolysis begins to decrease after only 4 hours, in *fructose* and *maltose* somewhat more rapidly; that it is quicker in maltose than in some monoses is remarkable.

Höfler is the first to discuss in detail in connection with deplasmolysis determinations, the possibility of anatonosis. He suggests a withdrawal of water by the sugars as the cause

of the decomposition of starch. (See also Molisch 1921, *a*). Although he does not prove it, permeation as the cause of the decrease of the plasmolysis, seems more probable to him than anatonosis. Nevertheless saccharose is, in view of the comparative slowness of the penetration, according to Höfler the best plasmolytic.

Bächer (1920) found, with the plasmolytic method, that cells of leaves of Elodea grown for 14 days on *saccharose* of 0.2—0.40 *n*, reached an *Oincip* as much higher as the osmotic value of the environment; after a longer time the value again fell somewhat. With Zea, cultivated on 0.10—0.30 *n*, he finds, after 6 days, a somewhat smaller rise. As to the cause, however, whether permeability or anatonosis, he expresses no opinion whatever; what he notes, however, is not without interest for me.

Fleischmann (1928) concluded from the decrease of the plasmolysis with calyx leaves of Hyacinthus that *glucose, fructose, maltose,* and *saccharose* permeate, saccharose the least so.

Finally I wish to mention, of those who investigated Phanerogamae leaves, Bärlund. Bärlund (1929) finds a value, with Rhoeo, of 0.01 *n* in *saccharose* after 24 hours, in *lactose* and *maltose* 0.02 *n* higher. He calls this a very slow permeation.

Summing up the experiments on Rhoeo we may therefore say that after 24 hours or sometimes less, some deplasmolysis occurs.

I will mention two investigators who worked with *Moulds*: Mayenburg (1901) saw no decrease after 5 hours with Aspergillus in *glucose* 37 %, Pantanelli (1904) found deplasmolysis on glucose after 12—14 hours, but did not conclude that permeability occurred. In themselves these two determinations have little significance, but they demonstrate clearly that the deplasmolysis depends on the time during which the plants remain in the solutions.

The investigations of Beggiatoa, a sulphur bacterium, by Ruhland and Hoffmann (1926), and Frl. Schönfelder (1931), and this will be the last publications to be cited, requires special mention. This was found to be very permeable both to *saccharose* and to *glucose*, and more so for the latter than for the former. I mention these publications apart because conclusions cannot be drawn from what is found with an organism of this kind as to its behaviour with Moulds, Algae, and Phanerogamae.

On summing up the deplasmolysis determinations it must first be noted that *by far the majority*, especially those carried out with Phanerogamae, *relate to a period of several hours, in some cases of several days. With most of the objects little or no deplasmolysis is found.* It is, of course, a mistake to conclude from this that no decrease will occur after a longer period, after weeks and months. (I cultivated my Nitella, for instance, for months in sugar).

As is seen from most of the publications, deplasmolysis is practically invariably taken to denote permeation. This conclusion is, of course, a very tempting one, as deplasmolysis is still more difficult to account for by means of anatonosis; it is, of course, quite within the bounds of possibility that the deplasmolysis is entirely or partly due to *permeation*, but this *is not proved by the deplasmolysis.* With salts, urea, etc., this might be done by means of chemical analysis of the cell liquid, by sugar and so forth to a certain extent also. In this connection attention may be called to the investigations of Schimper and Pfeffer (p. 303), which render permeation of glucose probable.

With Nitella translucens deplasmolysis experiments are practically impossible, since, if anything like a proper plasmolysis has occurred, the cells do not altogether recover. This does not tally with what Jost (1929) observed with Chara coronata, which remained alive for a long time in a plasmolysing solution of $CaCl_2$.

c. Anatonosis.

By anatonosis is to be understood an increase of the osmotic value in the cell (vacuole) through active new-formation or, as the case may be, decomposition of less osmotically active substances already present. It goes without saying that many of the writers also mention the possibility of permeation in their investigations. I wish, of course, to confine myself here principally to investigations with sugars; only occasionally will it be necessary to say something of other substances in order to make clear the idea of "anatonosis".

Investigations have been made with Moulds, Algae, and higher plants; as these organisms differ so widely in construction and behaviour, I will deal with the experiments as far as possible separately.

Investigations as to the osmotic adaptation of Aspergillus niger and Penicillium glaucum were made by Eschenhagen (1889). He cultivated spores in 0.5 % $NaNO_3$ plus *glucose* of 10—50 %. The "surplus", i.e. the difference between the osmotic value of the Mould cells and of the environment was found in every case to become greater according as the glucose solution was made stronger; with a glucose concentration higher than 40 % this difference remained practically the same. The osmotic value of the cells (*Oincip*) was determined plasmolytically. When the Moulds were transferred from this higher concentration to a lower one, the *Oincip* fell again in a few hours, again reaching the higher value in a several hours when they were again put into the strong glucose solution. In connection with this I would point out the fallaciousness of the argument that, when deplasmolysis occurs in solutions, this points to permeation, *because it proceeds so rapidly*. Eschenhagen's experiments, which practically prove anatonosis, render this argument invalid. Iljin (1923) also points out that an increase of turgor

through the formation of osmotically active substances may occur within a very short time.

Eschenhagen does not deny the possibility of permeation of the glucose or glycerol (with which he obtained similar results), but it is impossible to account in this way for the entire increase in the value, which is much greater than the increase of the substratum concentration.

He tested the cells, both macro- and microchemically, by means of the Fehling solution for glucose, but *did not find a trace of sugar*, so that an accumulation of sugar was out of the question. Moreover, in the Moulds which had reached such a high concentration in salt solutions he was practically unable chemically to demonstrate the presence of these salts. Eschenhagen therefore supposes that the high substratum concentration exerts an influence on the protoplasm, the result of which is a change in the metabolism, an anatonosis, that is. Exactly how this occurs, and what the osmotically active substances which are formed are, the writer is unable to say.

Pantanelli (1904) confirms Eschenhagen's experiments in practically every respect; he even finds a somewhat greater increase still of the osmotic value. His investigation is less complete, owing to his not having made any chemical tests, so that little value can be attached to his conclusion that anatonosis takes place.

The investigations of Von Mayenburg (1901) run parallel to the two preceding ones. Aspergillus cultivated in 55 % *glucose* undergoes a very great increase in osmotic value. With Fehling solution he obtained very little precipitate; he also attempted to show the presence of reduced sugar quantitatively (titrimetrically); the quantities he found were about 10 % of the increase in the osmotic value. Too much importance cannot, however, be attached to this, as it was impossible to get the Moulds quite dry; moreover, glucose may have been present in the protoplasm and in the

cell-wall. Von Mayenburg also determined the dry-weight in percentage of the fresh weight of dextrose cultures, and found this to be 43.7 % as against 15.3 % with normal cultures. The constituants of the ash had practically nothing to do with this increase, the cause very probably being organic substances, according to his analysis, however, not nitrogenous compounds, nor free organic acids. With Moulds cultivated in 50 % glucose he found 2 % glucose, which might therefore at any rate, partially, be responsible for the increase. Von Mayenburg supposes this to be due principally to "leicht zerfallende Zucker"; the most important cause of the rise in osmotic value he supposes in this case also to be a stimulating activity of the highly concentrated culture solution.

The investigation of Raciborsky (1905), as well as that of Laurent (1890) are of little value for my purpose. The former cultivates various Moulds in 25 % *glucose*, 50 % *saccharose*, and in saturated solutions of salts, amongst others NaCl (375 atmospheres); he makes neither determinations of osmotic value nor chemical tests — his researches only show that the Moulds grow in the solutions, so that his conclusion "anatonosis" is therefore not to be accepted without further evidence. Laurent demonstrated that yeast grows in glucose 55 % (about 70 atmospheres). Hawkins (1916) finally, found that various Moulds grew very well indeed in *glucose* to 2.6 *n* and in *saccharose* to 1.8 *n*.

Much less work has been done on the osmotic adaptation of *Algae*.

Van Rijsselberghe (1898) found (plasmolytically determined) increase of osmotic value of Spirogyra cultivated in *glucose* and *saccharose* of various concentrations; only a few days were required to reach this increase. The surplus became greater to a certain point, but decreased again with still greater concentration of the substratum.

Buchheim (1914) cultivated Cylindrocystis and Spiro-

gyra in NaCl and *saccharose* 0.9—7.2 %. They increased their osmotic value, this increase being attained in as little as 2 days; after 8 days the cells had retained the same value. According as the solution in which the Algae were immersed was more concentrated, the excess became smaller. Buchheim expresses no opinion as to the cause of the increase.

Finally there are a few earlier investigators who determined the osmotic value of higher plants in sugar, salt, and glycerol solutions. Stange (1892) put stems of various plants into Knop's solution, to which were added glycerol or salts of various concentration. Although, therefore, no sugar was used, it seems to me desirable to mention this investigation, in which an anatonosis is shown to be probable. In these cases, therefore, the cells tested did not come into direct contact with the solution. There was a great increase in osmotic value, and, as was also found by Van Rijsselberghe, Eschenhagen, Pantanelli, and Von Mayenburg, an "Ueberregulierung" took place. Permeation is therefore not very probable, at any rate the entire increase cannot be accounted for in this way; according to Stange there may be a conversion of the substance in the solution into osmotically more active substances.

Van Rijsselberghe (1898) determined the increase in the osmotic value of cells of Spirogyra (see p. 309), and of epidermis cells of Tradescantia and other plants in *glucose*, *saccharose* and *salts*. He again made use of the plasmolytic method. The results were the same as those mentioned above in the case of Spirogyra. He asks himself whether permeation or anatonosis is the cause of the increase. He was unable to find saccharose in the plants cultivated in that sugar (method of Trommer, Molisch), nor could he find glucose in the leaves put into glucose solutions (the method of Fehling, Ost). In the cells of leaves cultivated in salt solutions, on the other hand, these salts could, however, be shown, so that Van Rijsselberghe concludes that per-

meation takes place in those cases. He did not, however, previously determine whether these salts were also present from the commencement. In the case of the sugars, on the other hand, he is inclined to assume anatonosis. Here, however, we come to a weak point. If, in solutions of sugars and salts the osmotic excess i.e., the difference between the value of the cell-sap and the value of the environment constantly becomes greater, which was the case with Van Rijsselberghe, anatonosis is more probable than permeation (alone), as is distinctly evidenced by Eschenhagen, Pantanelli, and Von Mayenburg. Although this great excess is found by Van Rijsselberghe both with sugars *and* salts, anatonosis with salt cultures is according to him out of the question!

I may mention a good example of anatonosis and katatonosis (the opposite of anatonosis) found by Van Rijsselberghe and also cited by Ruhland (1915, *b*, p. 97) and Höber (1926, p. 413). In strongly hypotonic salt solutions crystals of calcium oxalate are formed in the cells of Tradescantia, which causes the osmotic value to decrease (katatonosis), and conversely the crystals disappear again in hypertonic solutions, thus causing a higher osmotic value by means of the formation of oxalic acid (it was possible to demonstrate this chemically). Anatonosis, therefore, took place here. Van Rijsselberghe supposes that, in the case of his cultures in saccharose and glucose these substances change in the protoplasm, decomposed into oxalic acid, amongst other things. That organic acids are in general of great importance for the turgor was observed by De Vries (1883, '97) and Pfeffer (1897, p. 121) as also by Ruhland (1915, *b*). Glucose, according to De Vries, plays a much smaller part.

Let us now consider the investigations into the formation of starch and the hydrolysis of this, also, that is, an anatonosis. As Nitella contains a great deal of starch, this possibility

is of great importance for my purpose. On the formation of starch from sugar I have, moreover made a number of tests (see p. 325).

Most investigators first free their objects from starch, they then put them into sugar solutions, and determine whether, after some time in the dark, starch can again be shown to be present. Some put their plants in the light, but remove the CO_2, which of course also makes the assimilation of carbonic acid impossible. The demonstration is always done with potassium iodide iodine or iodine in chloral hydrate.

I think it best, as I worked with Algae myself, to keep the publications relating to this group, which are not very numerous, and those relating to higher plants as far as possible apart. (For the latter I refer for the greater part to others).

Klebs (1886—'88 p. 538), made tests with Zygnema. If these Algae are placed in water in the dark, they lose their starch after 8 days, as they do also in saccharose solutions, but then only after 4 weeks. In the dark starch is again formed by Zygnema in 1—20 % *saccharose*. As the cells remained alive much longer in the dark in a sugar solution than in water, he supposes that the sugar is, after all, taken up.

The next to occupy himself in detail with this phenomenon is Bokorny (1918), who found formation of starch in Spirogyra, in *lactose*, *galactose*, *raffinose*, and *saccharose*, but not in *fructose*, as also in the case of Vaucheria (if carbonic acid is excluded). The same writer further states that he found no starch in 48 hours in the dark in 1 % *saccharose* in the case of Spirogyra, nor in 1 % *glucose* either. Bokorny's tests are not very complete so far as the indication of duration and concentration are concerned.

In the case of Bryum Pfeffer (1886—'88, p. 320) noted the formation of starch in plasmolysing (10—20 %) and non-plasmolysing solutions of *glucose*.

On various Phanerogamae, in most cases leaves, there are also many data of other investigators. For data on this point I may refer to the literature cited by others, e.g. Tollenaar (1925) and Weevers (1931).

Only two writers say anything as to the varying degree to which starch is formed in various sugars — both remarks refer to leaves of Beta.

Meyer (1886, p. 111) says: "Auf 10 % *Saccharose* wird schneller und vorzüglich reicher Stärke gebildet als auf einer 10 % *Dextrose*lösung".

Ruhland (1912, p. 225) observes that the starch is formed somewhat more rapidly in *cane sugar* and that the reaction is stronger than on *monoses*, "wohl infolge seines grösseren Moleküls, da er an und für sich etwas langsamer als jene permeiert". In this connection I would refer the reader to my own tests with Nitella on p. 325.

Tollenaar (1925) traced the formation of starch at various temperatures in the dark in the case of leaves of Nicotiana and Tropaeolum. Here, too, he obtained a positive result. With Tropaeolum he finds a maximum formation of starch in 7½ % *glucose* and in 14 % *saccharose* solution (both, that is, solutions of about 0.4 *n*). Plasmolysis prevents to some extent the formation of starch. He further found more saccharose, in the case of leaves lying in saccharose, than glucose in the leaves lying in glucose.

With regard to the *conditions under which starch is formed* and *under which it is decomposed*, a very extensive literature has been published; one cannot, unfortunately, get a clear idea from this as to the exact course of these processes.

The great majority of these investigations relate to the guard cells of stomata. For the literature I refer to Iljin, Weber and others.

So it is seen that increase of the osmotic value in sugar occurs both in Moulds (very strongly) and in Algae and higher plants. More especially the investigations of Eschenhagen

and of Von Mayenburg with Aspergillus make anatonosis very probable: a great increase of osmotic pressure is found, whilst little or no sugar can be found chemically.

II. My Investigation with Nitella.

1. *Cultures in Saccharose.*

The object was to determine whether Nitella cells in saccharose solutions of various concentration adapt themselves to their new environment so far as the osmotic value is concerned, and if so, whether that increase in concentration in the cells is caused by permeation of sugar into the cell or whether the cell in some way or another begins *actively* to form osmotically active substances (anatonosis).

The Algae were cultivated in glass dishes which were covered with a glass lid, except for a very narrow chink. The solutions were renewed every 3 days, the entirely or partly dead cells being at the same time removed; the cultures stood in the same laboratory room as the water cultures. For the purpose of checking, determinations were made with Nitellas in water.

The Algae were cultivated in saccharose solutions in main-water of 0.10 *n* and 0.20 *n*. In saccharose 0.30 *n* the cells soon died, both when they had an osmotic value of approx. 0.26 *n* and the sugar therefore plasmolysed, and when, in the winter of 1929, the value was about 0.50 *n* and no plasmolysis therefore occurred. In the solutions I used I was able to keep the Algae good for about two months.

Tables 21 and 22 show the result. In the first column is found the date on which the determinations were made, in the second, the value which the cells had in water during the time that determinations with sugar-cells were being made („water"). These control water-values are the averages of 2—4 determinations, which are to be found in Table 15*b*. In the column under „Days" is the number

of days that the cells of which determinations were being made had already been in saccharose. In the following column are found the osmotic values of the vacuole sap of the cells which had been cultivated for a certain number of days in 0.10 *n* saccharose, this number of days being found in the third column. After the values found is the average of these. The same is found in the last column of saccharose 0.20 *n*. It should be pointed out that Algae were put into the sugar on different days, so that I worked with several series which have been put together in this table.

Some of the figures are marked with *; these are the osmotic values of cell-sap obtained by mixing the sap of 3—4 cells and making the determinations with this. The accuracy of such determinations is, of course, greater than the value of one cell.

The standard deviation of the average of two or three values determined on one day will sometimes, of course, be fairly large; this will, however, be largely compensated for by the fact that determinations were made on so many successive dates, a fairly regular increase of the value being seen to occur with the „sugar-cells". This should also been borne in mind when considering the graphs.

In table 21 we see that the cells in *0.10 saccharose* begin to show an increase in osmotic value after about a week, the maximum value being reached October 5th (0.40 *n*, corresponding to about 11.1 atm.), which value remained constant for at least 2 weeks (see Oct. 18th). On Oct. 30th we again see, however, a rise (0.46 (13 atm.), then 0.52, i.e. 15 atm.).

In table 22 we see that the same thing happened in 1930, a rise to 0.37 *n* (10.1 atm.) (18 Sept.), this value remaining constant for at least 16 days (Oct. 8th), after which, as in 1929, a new increase took place (0.41 $n = 11.4$ atm.), which, however, could not be followed any further, as I had no more Algae at my disposal.

The cells in 0.20 *n* saccharose show first a rise to 0.41 *n*

TABLE 21

Date	Water	Days	Sacch. 0.10		Sacch. 0.20	
			Values	Average	Values	Average
27 Aug. '29	0.27					
7 Sept. '29	0.27					
12 „ '29		1	0.25, 0.25, 0.24, 0.26, 0.26, 0.26	0.26	0.30, 0.32, 0.31	0.31
13 „ '29		2	0.26, 0.26	0.26		
14 „ '29		3	0.24, 0.22, 0.26, 0.25, 0.26, 0.26	0.25	0.32, 0.29	0.31
17 „ '29	0.26					
24 „ '29		6	0.32, 0.33, 0.33	0.33	0.32, 0.32, 0.31	0.32
19 „ '29		8	0.34, 0.35	0.35	0.35, 0.34	0.35
27 „ '29		15	0.35, 0.32, 0.34	0.34		
30 „ '29	0.27					
5 Oct. '29		17			0.40, 0.41	0.41
5 „ '29		23	0.40, 0.40	0.40		
18 „ '29		30	0.40, 0.38	0.39	0.41, 0.43	0.42
18 „ '29		36	0.40, 0.39	0.40		
19 „ '29	0.26					
23 „ '29	0.27					
30 „ '29		41	0.47, 0.46, 0.46	0.46		
31 „ '29		42			0.46, 0.48, 0.47	0.47
3 Nov. '29	>0.34					
6 „ '29		49	0.52, 0.52	0.52		
8 „ '29	>0.38					
9 „ '29	>0.46					
11 „ '29	0.50					
12 „ '29	0.49	55	0.51, 0.53, 0.53	0.52		
17 „ '29		60	0.52	0.52		
27 „ '29	0.60					

Osmotic value of cells grown in saccharose solution, compared with that of cells cultivated in water.

1929

TABLE 22

Date	Water	Days	Sacch. 0.10		Sacch. 0.20	
			Values	Average	Values	Average
11 Aug. '30		4			0.30	0.30
15 „ '30		3	0.26	0.26		
15 „ '30		8	0.31	0.31	0.29	0.29
16 „ '30	0.27					
19 „ '30		12	0.29	0.29	0.33, 0.34, 0.33	0.33
20 „ '30	0.26					
20 „ '30		13	0.31*, 0.30	0.31	0.33*, 0.34	0.33
3 Sept. '30		23			0.36, 0.39	0.38
3 „ '30		27	0.34, 0.35	0.35		
8 „ '30	0.28	32	0.34, 0.35, 0.33	0.34	0.41	0.41
11 „ '30	0.26					
18 „ '30	0.25	38				0.44
18 „ '30		42	0.37*, 0.38	0.37		
20 „ '30		40			0.45*	0.45
30 „ '30		49	0.37*, 0.36	0.37		
30 „ '30		50			0.46, 0.47	0.47
1 Oct. '30	0.29[1])	51			0.47, 0.48, 0.47	0.47
6 „ '30		56			0.48	0.48
8 „ '30		58	0.36*, 0.37	0.36		
17 „ '30	0.26					
18 „ '30		66	0.41, 0.41	0.41		
20 „ '30	>0.30					
23 „ '30	0.31					
26 „ '30	0.34					

Osmotic value of cells grown in saccharose solution, compared with that of cells cultivated in water.
1930

[1]) On October 1st the value of one cell only was determined in water, this value 0.29 *n* being very probably higher than the average value of the cells on that date, since before and after that day a value of about 0.26 *n* was found.

= 11.4 atm., (Oct. 5th) [1]) in 1929 (table 21). The same value was still found on Oct. 18th (0.42 *n*). On October 13th, however, the value had risen to 0.47 *n* (13.3 atm.), a fresh rise having then taken place.

In 1930 (table 22) the value rose to 0.48 *n* (13.6 atm.) (Oct. 6th); after this determination the cells had all been used up.

In chapter C (table 15*b*) we have seen that, with cells grown in water, an autonomous increase of the osmotic value occurs in autumn. In tables 21 and 22 these values are again shown under the heading „Water", to allow of a comparison being made with the „saccharose values" on the same date. The high winter values are printed in heavy type in all the tables. The second rise in the values in the saccharose solutions was found to occur at the same time when the periodical rise of the cells in water began. These values are likewise printed in heavy type from the moment that this second rise began. *The cells in saccharose solutions thus also and simultaneously experienced these unknown autonomous influences.*

The comparison of the values in saccharose with those at the same time in water further shows that in the time in which the osmotic value in water remains approx. 0.26, the value in cane sugar solutions becomes higher; *the osmotic values adapt themselves to the environment.*

In 0.10 *n* saccharose the value of 0.26 *n* is increased to 0.40 *n* in 1929 (Oct. 5th), in 1930 (Sept. 18th) to 0.37 *n*; this is thus a complete adaptation to the environment, in 1929 even a „super-adaptation".

In 0.20 n saccharose solution the value of 0.26 *n* is increased to 0.42 *n* in 1929 (Oct. 5th), in 1930 (Oct. 6th) to 0.48 *n*, in 1930, that is, a complete adaptation, in 1929 not quite complete.

[1]) The slight increase to which the osmotic value is subject immediately after the cells have been put into a stronger solution e.g. 0.20 n, is of course largely due to a diminution in the cell-volume.

It should further be pointed out that in the winter of '29/'30, when the cells in water had a value of 0.50 *n* (November 12th), the value in 0.10 *n* saccharose was practically no higher than in water, the water-cells had, as it were, „caught up with" the sugar-cells. It seems therefore that cells which already possess such a high value resist an increase of the osmotic value when in the sugar solution. This is also supported by the determinations made in January, 1930, which are given in the following table 23.

TABLE 23

Date	Days	0.10 sacch. 25° C.	Water 25° C.
1 Jan. '30.....	5	0.62, 0.61	0.61, 0.61, 0.60
22 „ '30.....	26	0.55*, 0.54	0.53*
24 „ '30.....	28	0.53*	

After 5 days there was still no increase to be observed, nor after 26 days, but then the periodic lowering of the osmotic value already began. It seems therefore as if the autonomous factor brings the osmotic concentration to a particular value (in table 21 to 0.50—0.52 on Nov. 12th '29, in table 23 to 0.53), independently of whether the cells are in water or in a sugar solution.

In figs. 3 and 4 the values found in 1929 and 1930 respectively are shown graphically; the coincidence of the periodical rise of the water-cells with the second (autonomous) rise in saccharose is very plainly seen. In fig. 4, moreover, the values found in glucose 0.10 *n* are given. (With regards to the determinations with glucose 0.10 and 0.20 *n* see below).

2. *Cultures in Glucose.*

Experiments with glucose (dextrose) 0.10 and 0.20 *n* were made in the same way as with saccharose. Those with 0.10 *n* were made from August to November 1930, those with 0.20 *n* from July to September 1931. Table 24 shows the

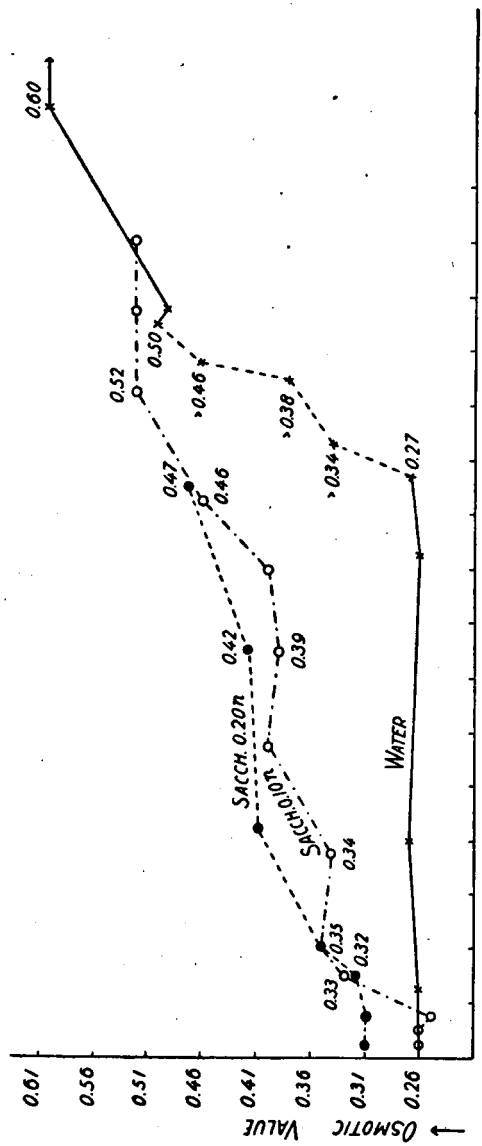

Fig. 3. The osmotic value in water and in saccharose 0.10 and 0.20 *n* September—November 1929. See table 21.

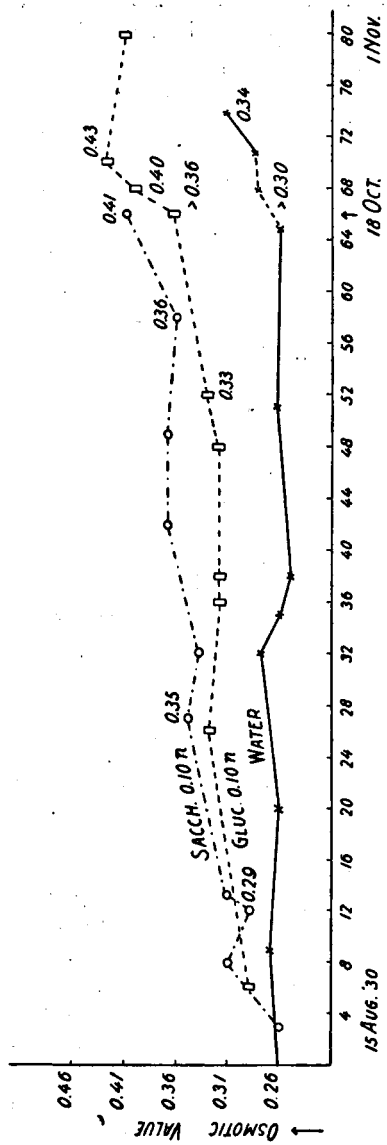

Fig. 4. The osmotic value in water, in saccharose 0.10 and in glucose 0.10 *n* August—November 1930. See tables 22 and 24.

result with 0.10 *n*, table 25 with 0.20 *n*. The graph of the values found with cells cultivated in 0.10 *n* is shown in fig. 4. The tables are arranged like tables 21 and 22.

TABLE 24

Date	Water	Days	Glucose 0.10 *n*	
			Values	Average
16 Aug. '30.....	0.27			
19 „ '30.....		6	0.29, 0.29	0.29
20 „ '30.....	0.26			
8 Sept. '30.....	0.28	26	0.33	0.33
11 „ '30.....	0.26			
18 „ '30.....	0.25	36	0.32	0.32
20 „ '30.....		38	0.31, 0.32	0.32
30 „ '30.....		48	0.32*	0.32
1 Oct. '30.....	0.29[1])			
4 „ '30.....		52	0.32, 0.33	0.33
17 „ '30.....	0.26			
18 „ '30.....		66	> 0.36	>0.36
20 „ '30.....	>0.30	68	0.40*, 0.41	0.40
22 „ '30.....		70	0.43, 0.42, 0.43	0.43
23 „ '30.....	0.31			
26 „ '30.....	0.34			
1 Nov. '30.....	0.33	80	0.41	0.41
3 „ '30.....	0.33			

Osmot. value of cells grown in glucose 0.10 *n* compared with that of cells cultivated in water. 1930.

TABLE 25

Date	Water	Days	Glucose 0.20 *n*	
			Values	Average
4 Aug. '31.....	0.26	29	0.37, 0.38*	0.38
19 „ '31.....	0.26	44	0.45, 0.44, 0.43	0.44
21 „ '31.....		46	0.43, 0.44*	0.44
1 Sept. '31.....	0.27	57	0.46*, 0.47	0.46

Osmot. value of cells grown in glucose 0.20 n, compared with that of cells cultivated in water. 1931.

[1]) See note p. 317.

From table 24 is seen that *in 0.10 n glucose* the increase in the osmotic value is about 0.05 *n* smaller than in cane sugar 0.10 *n*. The value 0.33 *n* (9 atm.), which was reached after 26 days, remained constant to October 4th, so that the cells only partially adapted themselves to the environment. On October 18th a fresh rise began, which it was possible to follow until November 1st (0.42 *n* = 11.7 atm.). The second increase was found to begin at the time that the autonomous periodical rise in value also set in (the high values are printed in heavy type). This new increase is therefore, as was also the case with saccharose, to be accounted for by the same unknown factor(s). See also fig. 4.

In table 25 we see that in 1931 *in 0.20 n glucose* complete osmotic adaptation came about.

3. *Influence of Temperature and Light on the Osmotic Value of Cells cultivated in 0.10 n Saccharose.*

There is practically no literature on this subject. Klebs (1886—'88) found that Zygnema in a saccharose solution was far less affected by darkness than when in water. He supposes that, when water is withdrawn, the metabolic processes, especially respiration, are retarded, and that the consumption of nutriment is consequently very slow. He made no experiments as to the influence on the osmotic value.

With Nitella I took no particular notice of whether they kept good for a longer time in the dark in saccharose than in water. I was, however, able to note that they remained alive for a very long time in the dark, at any rate in winter in main-water, which of course contains few nutritious constituents (from Dec. 18th '29 to Feb. 10th '30, i.e. 54 days; see table 16, p. 295).

Stange (1892) made, it is true, no experiments in this domain with sugar, but he did do so with glycerol. With Lupinus and Phaseolus he found that the osmotic value in the dark went up about 10 % less than in the light. He suggests various possibilities.

1. Greater longitudinal growth and no formation of osmotically active substances.
2. Assimilation is not possible in the dark. Substances already present are used up.
3. Less absorption of the substances available.

My own investigation with Nitella yielded the following result. In the summer of 1930 Algae were put into 0.10 saccharose in the dark, partly at 20 deg. C., partly at 1 deg. C. (in a refrigerator). Table 26 shows the values. Just as in the previous tables values marked with * were obtained from cell-sap from 3—5 cells which was mixed.

TABLE 26

Date	Days	Sacch. 0.10 *n* Dark, 20° C.	Sacch. 0.10 *n* Dark, 1° C.
9 Aug. '30.....	11		< 0.28
19 „ '30.....	12	0.28	
19 „ '30.....	21		0.30, 0.29*
3 Sept. '30.....	27	0.29*, 0.30	
3 „ '30.....	36	0.29*	0.28*, 0.29
8 „ '30.....	41		0.28

If we consider, that in water, in the dark, the osmotic value of these summer-cells at 1 deg. C. is 0.28 *n* (7.5 atm.), and at 22 deg. C. 0.27 *n* (7.3 atm.) (see table 19 p. 296), we must conclude that the increase in value both at 20 deg. and at 1 deg. is practically nil. This holds good of summer-cells, which have a lower value. In the winter of 1929, when the increase was extraordinarily high, 0.60 *n* (17.8 atm.), a small increase was found to occur in the dark at 25 deg. (table 27). It should, however, not be lost sight of that this is shown by a few observations only. At 9 deg. no increase was found. The values given at the bottom of table 21 and in table 23 render it further probable that with cells with a high value little or no increase occurs in the light either. It should further be pointed out hat the cells

which were put into the sugar solution on November 26th '29 had already been in the water for a fortnight at the same temperature and in the dark, so that they were already accustomed to their temperature.

TABLE 27

Date	Days	0.10 Sacch. Dark, 9° C.	0.10 Sacch. Dark, 25° C.	Water Dark, 9° C.	Water Dark, 25° C.
27 Nov. '29	1	0.62, 0.63, 0.61	0.56, 0.55	0.61, 0.60	0.55, 0.54
29 „ '29	3	0.64	0.56	0.62, 0.60	0.55, 0.55, 0.56
4 Dec. '29	8	0.64	0.58, 0.61	0.61*, 0.62	0.56
14 „ '29	18	0.64, 0.63	0.60, 0.58	0.62, 0.60	0.55*, 0.55
22 „ '29	26	0.61, 0.62		0.59*	

From table 27 it is further seen that, at 9 deg. in the dark in 0.10 *n* saccharose the value is about 0.04 *n* (1.06 atm.) higher than at 25 deg.; that this, however, has nothing to do with the sugar is seen from the last two columns — this difference also occurs in water, so that the difference in temperature is the sole cause.

The observations here discussed render it very probable that no increase occurs in the case of summer-cells in the dark in 0.10 *n* saccharose (I made very few determinations on this point: tab. 26), and that in the case of winter-cells with a high value the osmotic concentration, which rises very little, if at all, in the light in 0.10 cane-sugar, does so very little in the dark also. The temperature seems to have very little influence here.

4. *Formation of Starch in Nitella.*

In connection with the researches into the formation of starch and the hydrolysis of starch in sugar solutions (see the summary of the literature p. 312), I carried out a few tests on these points with Nitella in the summer of 1931.

In normal cells a great deal of starch occurs, but after a few weeks in the dark it disappears in the great majority of cells.

A number of Algae which had been freed in this way from starch were put into *saccharose 0.10 n* and into *glucose 0.10 and 0.20 n* for 9 days in the dark. In the table given below the number of cells which on the date mentioned contained a great deal of starch, is found under + + +. Macroscopically, cells treated for several days with alcohol at once became black on the addition of potassium iodide iodine. Under + + is found the number of cells which became much less black, although this was noticeable macroscopically, and very evident microscopically; under + the cells in which starch could only be shown to be present microscopically, and under — the cells which were microscopically quite free from starch. On the 17th of August 13 cells were investigated in the light. The rest were then placed in the dark in water, and on each of three dates a number of cells were tested for starch. On Sept. 8th the remaining Algae were put into the sugars and tested on Sept. 17th.

TABLE 28

Cultivating solution	Date	% of cells +++ and ++	+++	++	+	—
Water—Light	17 Aug., '31	100	12	1		
„ —Dark	21 „ '31	66	4			2
„ — „	3 Sept., '31	17		1	2	3
„ — „	8 „ '31	14	2	2	3	23
Glucose 0.10—Dark....	17 „ '31	30	1	2	2	5
„ 0.20— „	17 „ '31	33	2	2	1	7
Sacch. 0.10— „	17 „ '31	61	4	5	2	2

We see in table 28 that, on September 8th, the majority of the cells are quite (—) or almost (+) free from starch: 86 %. After being kept in the dark for 9 days in 0.10 *n* glucose, 30 % again contain a great deal (+ + +) or a good deal (+ +) of starch; 33 % in 0.20 *n* glucose, and 61 % in 0.10 *n* saccharose.

These figures render it probable that starch can be made from glucose, while it is practically certain that this is the case in saccharose. Klebs points out with respect to the same tests that a great many cells are required; I had, however, no more cells at my disposal, and had therefore to make shift with a comparatively small number. A repetition and extension of my experiments is therefore desirable.

In 0.10 *n* saccharose twice as much starch is formed as in 0.10 *n* glucose, but it should not be overlooked that the concentrations expressed in *percentages* are 3.4 % saccharose and 1.8 % glucose, that of the saccharose being thus twice as strong. When we read that Tollenaar (see p. 313) found a *maximum* formation of starch in 7½ % glucose and in 14 % saccharose (both, that is, about 0.4 *n*), it must not be concluded from my tests that more starch is formed in saccharose than in glucose, although according to several authors (Meyer, Ruhland, see p. 313) this is not impossible.

In August, 1931, I determined the content of starch of cells which had been cultivated for 1 month in the light in 0.2 *n* glucose and in 0.1 *n* saccharose. I found them to be as rich in starch as before they were put into the sugar, at any rate not less so. I made these tests because it is, of course, not altogether impossible that the increase in the osmotic value may be due to hydrolysis of the starch present; although the experiments of Iljin and others are not all equally clear and do not always agree with one another, and hydrolysis of starch in such weak sugar concentrations as I used (0.1 *n*) does not, on the strength of their experiments, seem very likely, the possibility of hydrolysis must at any rate be considered. From the fact that, a month later, when a very considerable increase of the osmotic value had occurred, I found as much starch as ever, it may be deduced that, when hydrolysis has taken place, this is certainly not the case to such a degree that the loss of starch cannot be com-

pensated for by fresh formation of new starch, whether by assimilation from carbonic acid and water or from the sugar of the solution in which the Algae are lying.

With regard to the cause of the increase of the osmotic value, see further the discussion at the end of this chapter.

5. *Sugar-tests of the Cell-sap of Nitella.*

In order to find out whether the increase in osmotic value in sugar solutions is to be attributed to anatonosis or to permeation of the sugar, it was of great interest to know whether sugar can be shown to be present in the cells. It is, of course, first necessary to know whether sugar is present in cells grown in water.

I now cast round for a micro-method of showing the presence of sugar, preferably quantitatively, in the cell-sap.

A few determinations were first made by way of preliminary test with the qualitative osazon method according to Senft[1]) (Molisch 1921, p. 131) with normal cell-sap from water-cells and with cell-sap of Nitella which, on Oct. 18th 1929 had lain for 30 days in saccharose 0.10 *n* and had an osmotic value of 0.39 *n* (10.8 atm.) (in water 0.26 *n*); these latter cells might thus be expected to show a positive reaction when the sugar as such had permeated. I made a solution of muriatic phenylhydrazine in glycerol of 1 : 10 and a solution of Na acetate in glycerol 1 : 10. A drop of the first solution was mixed with a drop of the second on an object-slide. A drop of the liquid which it is desired to test for sugar is then added. When this is heated over a water-bath any bioses which may be present are inverted by the glycerol, so that the glucose formed shows the osazon reaction. The results were as follows.

[1]) There are, as is well-known, a number of other methods, some of which are more accurate than the osazon method; the qualitative tests mentioned here were, however, only intended as preliminary tests.

0.1 *n* glucose solution at once gave a strong + reaction both cold and after heating.

0.1 *n* saccharose solution gave a + reaction a few weeks after heating.

These two control-tests therefore proved the reliability of the method. I then made a test with vacuole liquid from water-cells and from sugar-cells as stated above:

Normal vacuole liquid cold and after heating, even after several weeks: reaction negative.

Vacuole liquid from „sugar cells" with a value of 0.39 n, the same negative result.

As, however, this method was merely qualitative, I proceeded to another one — the *micro-fermentation method of Van Lutsenburg Maas—Van Iterson* (1915) [1]), which as far as I am aware, has never been used before in botanical investigations, and which proved eminently satisfactory. The principle of this method, which is a micro-modification of that of Kluyver (1914), is as follows.

A glass capillary, about 20 cm. long, which is divided into $^1/_{100}$ cc. is partly filled with mercury, a drop of yeast emulsion is then put into the capillary, coming into contact with the mercury, and next a drop of the liquid which it is desired to test for sugar. If desired, kinds of yeast may be taken which ferment only a particular kind of sugar. I made in all cases use of Saccharomyces cerevisiae, which ferments both glucose and saccharose. The height of the mercury is read off before the yeast is put in, after this has been done and finally once more when the sugar solution has been added. An estimate is made to $^1/_{10}$ of a line on the scale,

[1]) This method curiously enough is not mentioned in Lehmann's monograph (1931), nor is it referred to by Miss Widdowson (1931). Keulemans (1928) made tests with extracts from leaves with a method of Van Iterson—Kluyver, which is based on the same principle, for which, however, more liquid is required; for 3 tests 5 cc which contain 30 mgr. monose. See further Gast (1917).

so that the volume of the yeast emulsion and of the sugar solution is known to $^1/_{1000}$ cc. The tube is then closed with paraffin at the end where the two drops were allowed to enter. The apparatus is then kept for 6 hours in a thermostat at 30 deg. C., being then brought to room-temperature, when the height of the mercury meniscus is read off. From the volume of carbonic acid produced, which is converted to 0 deg. C. and 76 cm. pressure (for which a table is to be found in Kluyver (1914, p. 61), the quantity of sugar is calculated, and, as the volume of liquid is known the percentage of sugar also. For the technique I would refer the reader to the publication by Van Iterson—Lutsenburg Maas.

There are several conditions which must be complied with: the quantities of sugar fermentable are between 3.5 mgr. and 0.1 mgr., a drop of 0.010 cc. sugar solution being sufficient; the concentration of the solution must be greater than 0.40 %. 1 cub. cm. CO_2 formed at 0 deg. and 76 cm. pressure corresponds to 4.05 mgr. anhydrous hexose and to 3.85 mgr. anhydrous biose (saccharose etc.).

By way of control a few tests were first made with a drop of saccharose 0.10 *n*, two examples of which are shown in the following table.

TABLE 29

Quantity of CO_2 produced	Corresponding to mgr. sacch.	Theoretical quantity of CO_2 to be produced	Corresponding to mgr. sacch.	Concentration found	Real concentration
0.535 cc.	2.066	0.540 cc.	2.086	0.099 n	0.10 n
0.213 cc.	0.824	0.221 cc.	0.854	0.096 n.	0.10 n

These two tests prove the accuracy of the method.

Normal cells.

On December 28th, 1929, 3 *normal cells* from water were tested, no carbonic acid being formed in any of the tests,

so that *the cell-sap cannot have contained any fermentable sugar*. These cells had a high winter value, 0.60 *n*; this, therefore, also proves at the same time that the periodical increase in value is not to be attributed to sugar. In these, as in the following determinations, sap from 2 or 3 cells was frequently required to allow of a cell-sap drop of the proper size being obtained.

Cells from saccharose 0.10 n.

In October '29 I made 5 tests with vacuole sap from cells which had been cultivated for 33—34 days in *cane-sugar 0.10 n* and which had a value of 0.39 to 0.40 *n* (11.1 atm.). In none of the 5 tests was a trace of CO_2 formed. The possibility remains that CO_2 was dissolved in the liquid (the yeast emulsion and the cell-sap), but, if so, this can only have been a very little. A number of other tests were, however, also made, in which, in addition to a drop of cell-sap a drop of 0.10 *n* saccharose was also put into the capillary; this must, of course, in any case yield a calculable quantity of carbonic acid. The next table 30 shows 3 determinations. In the first column is the amount of sugar present in the sugar solution plus the cell-sap, calculated from the volume of carbonic acid formed; in the second column the amount of sugar which had dissolved in the sugar-drop. In the third column is the concentration calculated from the sugar found (that is, calculated from the figures in the first column), and finally in the last column the actual concentration of the sugar drop: 0.10 *n*.

The concentrations of the sugar in the drop of sugar

TABLE 30

Mgr. sacch. calculated from the CO_2	Real quantity of sacch.	Concentration found	Real concentration
0.594	0.614 mgr.	0.098 *n*	0.10 *n*
0.687	0.717 mgr.	0.096 *n*	0.10 *n*
0.742	0.802 mgr.	0.093 *n*	0.10 *n*

solution plus the vacuole-sap which I found were all three several thousandths *n* smaller than the actual concentration of the drop of sugar alone, 0.10 *n*. *There was therefore absolutely no sugar in the cell.*

On September 24th, 1930, 2 further tests were made as above with cells which had lain for 44 days in a solution of 0.10 *n* saccharose and which had a value of 0.37 *n*; the result is shown in the following table. In the first column is the number of cc. CO_2 formed, in the next one the number of cc. CO_2 that should theoretically have been formed by the sugar from the sugar-drop, and in the last column the difference between the amounts of carbonic acid found and calculated theoretically.

TABLE 31

cc. CO_2 produced	cc. CO_2 calculated from the sugar	Difference
0.109	0.106	0.003 cc.
0.111	0.114	— 0.003 cc.

Practically, therefore, no CO_2 is left for the drop of vacuole liquid either, so that in this case also the conclusion must be come to that no sugar was present in the cell-sap. I made a further determination on Sept. 26th '30 with vacuole-liquid alone, no CO_2 being formed.

It should further be noted that these tests, in which cell-sap plus sugar was used, at the same time prove that cell-sap has not deleterious effect on the yeast; for exactly as much CO_2 is formed as corresponds to the sugar in the sugar drop.

Cells from saccharose 0.20 n [1]).

[1]) In the following 8 tests in tables 32 and 33 are several in which the minimum volume of the cell-sap laid down by Lutsenburg Maas—Van Iterson (viz. 0.010 cc.) was not reached; this naturally diminishes the accuracy of the tests, but will have little effect on the average value of the concentration.

Tests with these were made at the end of September, 1930, when the cells had been lying for about 50 days in the solution and had an osmotic value of 0.47 *n* (13.3 atm.), 0.20 *n* higher, that is, than in water. 4 tests were made with cell-sap alone. Carbonic acid was now actually found to have been formed, but nothing like so much that the rise in value, which amounted to about 0.20 *n*, could be accounted for by means of the sugar, which the CO_2 had formed. The sugar calculated from the carbonic acid formed was found to be present in the cell-sap in the following concentrations.

TABLE 32

Volume of cell-sap drop ...	0.005 cc.	0.016 cc.	0.004 cc.	0.010 cc.
Concentration of sugar found (calculated on saccharose)	0.04 *n*	0.06 *n*	0.02 *n*	0.02 *n*

In addition to the above, 4 tests were made with a drop of cell-sap plus a drop of saccharose 0.10 *n*. With these more CO_2 was formed than the sugar in the sugar drop could have produced alone.

TABLE 33

Volume of cell-sap	0.013 cc.	0.011 cc.	0.013 cc.	0.007 cc.
Volume sacchar. 0.10 n	0.012 cc.	0.021 cc.	0.034 cc.	0.006 cc.
Total amount CO_2 produced	0.144 cc.	0.210 cc.	0.314 cc.	0.050 cc.
Amount of CO_2 produced by the saccharose from the saccharose drop	0.106 cc.	0.185 cc.	0.299 cc.	0.053 cc.
CO_2 remaining for sugar from the cell-sap	0.038 cc.	0.025 cc.	0.015 cc.	-0.003 cc.
Concentr. of the sugar (calculated on saccharose) in the cell-sap, calculated from above amounts of CO_2 ...	0.03 *n*	0.03 *n*	0.01 *n*	0.00 *n*

The above two tables show that *the sugar concentration of the vacuole liquid averages 0.026 n, that is, 13 % of the total increase in value (0.20 n).*

Cells in 0.10 n glucose.

Three determinations were made at the end of September, 1930, when the value of the cells after these had been in the solution for 50 days was 0.32 *n* (8.7 atm.), 0.06 higher, that is, than the normal value; 1 test was made with vacuole liquid alone — no CO_2; 2 tests were made with vacuole liquid plus a drop of saccharose 0.10 *n*.:

TABLE 34

Volume of the cell-sap	0.022 cc.	0.011 cc.
Volume sacchar. 0.10 *n*.....	0.018 cc.	0.011 cc.
Vol. CO_2 produced	0.153 cc.	0.103 cc.
Vol. CO_2 produced by the sacch. 0.10 *n*.	0.158 cc.	0.097 cc.
CO_2 remaining for the cell-sap	−0.003 cc.	0.006 cc.
Concentr. sugar in the cell-sap	0.00 *n*	0.006 *n*

From these 3 tests it may be concluded that very little, if any, sugar (calculated on glucose) is present.

Cells in 0.20 n glucose.

Three determinations were made on August 21st, 1931, when the cells had a value of 0.44 *n* (12.4 atm.), i.e. 0.18 *n* (4.7 atm.) higher than in water, and 3 tests on Sept. 1st '31, when the value was 0.46 to 0.47 *n* (13.3 atm.), i.e. 0.20 *n* (5.3 atm.) higher than normal [1]). A drop of 0.10 *n* saccharose was added to the drop of vacuole liquid.

The results of the tests are shown in the following table.

[1]) As only very few tests were made with the cells in 0.20 glucose, the sugar concentration found must not be regarded as being absolutely exact.

TABLE 35

Volume of the cell-sap	Aug. 21st Osm. val. 0.44			Sept. 1st Osm. Val. 0.47 n		
	0.026 cc.	0.020 cc.	0.014 cc.	0.019 cc.	0.007 cc.	0.029 cc.
Vol. of the saccharose 0.10 n	0.009 cc.	0.017 cc.	0.024 cc.	0.013 cc.	0.020 cc.	0.030 cc.
Volume of the CO_2 produced	0.181 cc.	0.256 cc.	0.280 cc.	0.249 cc.	0.239 cc.	0.482 cc.
Vol. of the CO_2 produced by the saccharose 0.10 n ...	0.079 cc.	0.150 cc.	0.211 cc.	0.114 cc.	0.176 cc.	0.255 cc.
Vol. of the CO_2 remaining for the cell-sap	0.102 cc.	0.106 cc.	0.069 cc.	0.135 cc.	0.063 cc.	0.227 cc.
Concentr. of the glucose in the cell-sap	0.09 *n*	0.12 *n*	0.11 *n*	0.16 *n*	0.20 *n*	0.17 *n*

The above table shows that in 0.20 *n* glucose the sugar was actually found, and in very considerable quantities in the cells. *On August 21st, when the cells had been in the solution for 46 days* and *the increase in value amounted to 0.18 n, 0.11 of this was to be ascribed to sugar in the cell* (calculated on glucose). On Sept. 1st, when the Algae had been in the sugar for 57 days and the rise in value amounted to 0.20 *n*, 0.18 *n* was due to sugar in the cell, by far the greatest part, that is.

Summing up, we come to the conclusion that:

1. in *normal water-cells* no sugar occurs,
2. cells which had been 33—34 and 44 days resp. in *0.10 saccharose* and which had attained a value of 0.40 *n* (11.1 atm.) and 0.37 *n* (10.1 atm.) resp., contained no sugar in the cell-sap,
3. in the case of Nitella which had been cultivated in *0.20 saccharose* and had an osmotic value of about

0.47 *n* (13.3 atm.), 0.20 *n* higher, that is, than in water, sugar could be shown to be present in the cell-sap, the concentration of which was on an average 0.026 *n*, i.e. 13 % of the total increase in value,

4. with Algae which had been cultivated for 50 days in *0.10 n glucose* and which had a value of 0.32 *n* (8.7 atm.), that is, 0.06 *n* higher than in water, no sugar could be shown to be present, and finally that,
5. with cells which had been cultivated for 46 days in *0.20 n glucose* and had attained an increase in value of 0.18 *n* (4.7 atm.), 0.11 *n* (2.9 atm.) of this is due to sugar (glucose), and that after 57 days, when the increase in value amounted to 0.20 *n* (5.3 atm.), 0.18 *n* of this is to be put down to sugar.

6. *Discussion of the Alternatives, Anatonosis and Permeation, as Causes of the Increase in Osmotic Value.*

In putting the question whether the increase is due to anatonosis or (and) to permeation, the tests made with saccharose solutions must be treated separately from those with glucose solutions, as the tests for sugar in the cell-sap had such divergent results.

Both in 0.10 *n* and in 0.20 *n* *saccharose* an increase in the osmotic value is seen to occur, which begins after a few days and reaches a maximum after 3—5 weeks; the cells had then adapted themselves practically perfectly to the environment, i.e. the increase in value was about the same as the osmotic value of the sugar solution in which the Algae were growing. So far as the rise could be followed no superregulation occurred, as in the determinations of Van Rijsselberghe (p. 309) with Spirogyra and with higher plants (Stange, Van Rijsselberghe).

If it is borne in mind that I was *unable* to show *any trace of sugar in the sap of Nitella cells which had been cultivated in 0.10 n saccharose* and had a value of from

0.37 to 0.39 *n*, and only so much *in cells cultivated in 0.20 n cane-sugar* and which had a value of 0.42 to 0.48 *n*, *as would account for only about 13 % of the total increase in value*, this rise cannot be accounted for by mere permeation of the saccharose. Nor can I regard as probable, on the strength of the literature, such a great degree of permeability of saccharose.

Three possibilities remain:

1. The saccharose solution acts merely as a stimulus, as a result of which the increased metabolism takes place, or molecules in the vacuole are broken up into smaller ones, so that the concentration is increased.
2. The starch in the cells is decomposed into osmotically active substances (acids).
3. The saccharose from outside penetrates into the protoplasm, and is there changed directly into osmotically more active substances (acids?), which find their way through the tonoplast.

The first mentioned cause, the description of which, including that of Lapicque (p. 302), is vague, cannot be ruled out altogether, but there is really nothing to support it [1]).

For the second possibility, decomposition of the starch into osmotically active substances, there is more to be said. The investigations of Schimper and Ahrns, who demonstrated that starch in the plants can be decomposed

[1]) That something of the sort may be the case with Moulds, at any rate, is seen from the culture experiments in glucose of Eschenhagen, Pantanelli, and V. Mayenburg (p. 308), who found a very marked rise in concentration, but were unable to show that there was any sugar in the cells. Eschenhagen speaks of an "influence of the high substratum concentration on the protoplasm, which results in a change in the metabolism"; V. Mayenburg supposes that „leicht zerfallende Zucker" are formed. It seems to me more likely that the glucose is changed in the protoplasm into osmotically more active substances.

into monoses are of little value to me in so far as I was able to show little or no sugar in the Nitella cells; if this decomposition occurs, the glucose formed must again fall immediately into other substances (acids?).

Nor does the mere fact that the withdrawal of water in mesophyll cells can cause starch to disappear (Ahrns, Molisch, Strugger and Weber, Iljin) account for the increase in the osmotic value in the Nitella cells, more especially as the starch did not even disappear. I do not feel justified in drawing any conclusion from the investigations with stomata guard cells.

We then come to the third alternative: the saccharose penetrates into the protoplasm and is at once changed into osmotically more active substances.

Van Rijsselberghe (p. 311) supposed this to be possibly the case with cultures in saccharose and glucose, and had in mind the formation of oxalic acid. Schimper (p. 303) supposed that something of the sort occurs with glucose, partly because this substance plays such a large part in the assimilation of the plants, and because glucose as such is not very permeable for the protoplasm, at least, not in a short time. That Moulds grow so well in glucose and saccharose (Raciborsky, Laurent, Hawkins — p. 309) also proves that substance is taken up in some way or other.

Although this possibility seems to me to be by no means out of the question, I have no proofs of it.

Summing up, therefore, I come to the conclusion *that the increase in value of the saccharose does not come about through permeation of the saccharose as such* (except, perhaps, in 0.20 *n* to a small extent (13 %), whilst it is not entirely impossible that this sugar is formed by decomposition of starch), *but that this is the result of anatonosis.* Probably part of the starch (via sugars?) is decomposed into osmotically active substances or the saccharose is converted into similar substances in the protoplasm; this

is (see also Van Rijsselberghe), however, also a kind of anatonosis.

Finally I would point out that the experiments mentioned on p. 325 render it probable that little or no increase occurs in the dark, so that the light seems to have something to do with it.

In my culture experiments *in glucose solution* the matter is, at any rate partly, a different one. In *0.10 n* glucose the value is increased by 0.07 *n* after a few weeks, at which height (0.33 *n*) the value remains for 52 days (see table 24, p. 322), when no sugar could be shown to be present by the Lutsenburg Maas—Van Iterson method. For the same reason as mentioned with the saccharose cultures I must here assume *anatonosis.*

In *0.20 n* glucose solution the value rises, after 44 days, to 0.44 *n*, this being, after 57 days, 0.46 *n*. Then, however, sugar could most certainly be shown to be present in the cell-sap (see p. 335). On August 21st, when the value was 0.44 *n*, 0.18 higher, that is, than in water, 0.11 *n* of this could be ascribed to sugar present in the sap, and only 0.07 *n* was to be accounted for by the above-mentioned anatonosis. On September 1st (value 0.46 *n*), that is 0.20 *n* more than in water, 0.18 of this could be accounted for by the sugar present in the cells. There is, of course, a possibility of decomposition of starch into sugars through the withdrawal of water, which is naturally stronger in 0.20 *n* than in 0.10 *n* [1]).

To my mind it is more probable that, in 0.20 glucose, *the sugar as such permeates,* the more so as permeability is much more to be expected of glucose than of saccharose (see summary of literature pp. 301 et seq.) *so that the increase in osmotic concentration in 0.20 n must to a great*

[1]) Also Weevers (1931) states that something like this may happen.

extent be attributed to this; when writing my former publication (Wildervanck 1931) I had not yet made these tests, for which reason I did not at that time consider permeation of glucose likely. It can easily be imagined that sugar permeates into the cell in 0.20 *n* glucose and not in the much weaker 0.10 *n* solution.

E. CULTURES IN UREA.

Urea, as is well-known, is the favourite substance for permeability studies with plants; it was one of the first substances with which permeability was shown to occur. As, moreover, this compound is a product of metabolism of various plants (see e.g. the monograph of Kiesel (1927) and Tunmann (1931), I thought it well worth while tracing its effect on Nitella.

I. Literature.

Hugo de Vries (1889) was the first to demonstrate decrease of plasmolysis in Tradescantia (Rhoeo); after 1 day or less he found deplasmolysis in 1.2 %—2.7 % urea, the osmotic value (*Oincip*) rising to twice the original value. Cells which first showed this in a saccharose solution of a particular concentration no longer did so after being for some time in urea, which, according to the writer mentioned, is „der direkte Beweis, dass durch Aufnahme von Harnstoff die plasmolytische Grenzconcentration erhöht worden ist". After 24 hours in the cane-sugar plasmolysis recommenced, the urea taken up, apparently diffusing again out of the cells.

De Vries observed that the permeability was not the same for all the cells of one plant, which was confirmed by Fitting (1920), with Hemerocallis by Höfler and Weber (1926). De Vries also observed deplasmolysis later with other higher plants. I must observe en passant that he did not make chemical tests with any of his

experiments. With regard to the values found in the case of Tradescantia by several investigators, I wish to mention: in 0.16 *n* after 4 hours the *Oincip* still the same, after 24 hours 0.03 *n* higher (De Vries); in 0.5 *n* urea 0.01—0.03 *n* permeates per hour (Höfler and Stiegler 1921); after 15 hours in an isotonic solution (about 0.2 *n*) the value rises by 0.008—0.016 *n* (Fitting 1920). On the difference in permeability (or, at any rate, the rise in osmotic value) between plasmolysed and non-plasmolysed cells Bärlund (1929) carried out simple but ingenious experiments. In both cases the same rise occurred. On this subject Fitting, for glycerol also, (1920), for glycerol and saccharose with Spirogyra Lepeschkin (1909), for salts Ruhland (1915, *a*), for glycerol De Vries (1888) also write. They all come to the conclusion that this makes no difference. A very marked permeability, or at least a rapid decrease of the plasmolysis, is shown by Gentiana Sturmiana: in 1 *n* solution plasmolysis occurred after a few minutes, after 5—14 minutes this had again disappeared; 0.02—0.07 *n* permeates per minute (Höfler and Stiegler); the permeability is according to them 170 times as great as for KNO_3 and with Rhoeo equally great. In cells of scales of Allium Cepa 0.04—0.11 *n* per hour permeates in a 1.0 solution (Höfler and Stiegler). Höfler and Stiegler carried out their determinations by means of the plasmometric method (see also Höfler 1918).

Overton (1895, p. 184) found plasmolysis with Spirogyra in 1 % urea after 5 hours; the molecules dissolved were permeable in both directions through the plasma, as had already been noted by De Vries ('89) with Rhoeo.

Höfler and Weber (1926) found a slow decrease of the plasmolysis of cells of Hemerocallis in 1.2 *n* (7 %) urea; they obtained values which agree with those found by Höfler and Stiegler and Fitting in the case of Tradescantia.

Beggiatoa, the sulphur bacterium which I have already mentioned in the summary of the literature of the experiments with sugar solutions, is found to be very permeable for urea also, at any rate the osmotic value is very greatly increased (Ruhland and Hoffmann 1926). The permeability is therefore seen to vary with different plants.

As I have already remarked, deplasmolysis is invariably held to indicate permeability, as is the case with other substances, and it does, in fact, render it very probable; but to my mind chemical determinations of the cell-sap, such as have been made with salts by various investigators, are required to give complete conviction.

Höfler and Weber (1926) ask themselves whether the rise in osmotic value may not come about owing to anatonosis („aktive Neubildung"); they reject this possibility, however, on the strength of the fact that no deplasmolysis is to be observed in plasmolysing sugar solution after hours, and if anatonosis had taken place in a urea solution, this would, according to the writers, also necessarily occur in saccharose. They say finally that „damit diese Denkungsmöglichkeit entfällt".

This argument is to my mind not very clear. Non-deplasmolysis in saccharose may just as well be held to indicate that no permeation takes place; moreover, it is not at all impossible that deplasmolysis actually did occur after a longer time. I myself found in the case of Nitella in saccharose solutions no increase of the osmotic concentration in the first few days; this did, however, occur after a week and longer. And then, different substances may act quite differently on plasmolysed cells; even if anatonosis occurs, e.g., in saccharose, this does not give one the right to conclude, by analogy, that this is also the case in urea. Iljin demonstrated furthermore that one salt increased the osmotic pressure in the case of stomata guard cells, whilst another did not; it may even depend on the concentration. And that

different *salts* do not have the same effect, so that the action of one salt is no guide to that of another, was also made clear by Strugger and Weber (1925).

Bärlund (1929), who carried out a very extensive investigation on urea permeability, found that the osmotic value of Rhoeo cells, after 24 hours in a urea solution, became as much higher as the degree of concentration of the solution (determined plasmolytically with sugar); in another series of experiments he found that the higher value reached by the cells in urea solution after 2 hours, then gradually decreased again, which he explains by exosmosis of the urea. Unfortunately he did not try to find out whether this value did not fall back to that of the solution itself and then in water to 0; I very often found this with my Nitella experiments (p. 354). This, of course, points to injury or even death of the protoplasm.

He rightly states (p. 49) that, if permeability is to be predicated, it must be possible to rule out exosmosis, anatonosis, and katatonosis if an increase of the osmotic value is found in urea solution. As the value did not decrease, but on the contrary increased, he therefore rules out exosmosis and anatonosis; and yet, if there is exosmosis *and* anatonosis, or endosmosis *and* katatonosis, in both cases a higher value may be found, provided the anatonosis is greater than the exosmosis, or the endosmosis greater than the katatonosis. No evidence therefore is to be found in his argument for permeability (endosmosis), however probable endosmosis, in many cases may seem to be rendered by deplasmolysis and an increase of the osmotic value.

Various writers have studied *the poisonousness of urea for plants*. As it occurs in some plants as an intermediate product of metabolism, it goes without saying that it is not poisonous in those cases, in which the concentration is slight. Two older publications (Hampe 1867, A. Beyer 1869) further show that some higher plants can take up

urea from a culture-solution, which substance can then be shown to be present in the leaves and stems. De Vries ('89) finds that Tradescantia cells in a solution which is not strong enough to cause plasmolysis 1.7—2.2 %, are still alive after 5 days; urea in this concentration is, however, not innoxious to Spirogyra. Nathansohn (1903) finds injury, in cultivation experiments with Codium tomentosum after 48 hours in 6.5 % (and also in glycerol and glucose, for that matter). Bokorny (1918) kept Spirogyra alive in 0.02 % (= 0.003 *n*) urea plus some inorganic salts for 4 weeks; this is, of course, an extremely weak solution. 1 % urea is, according to Bokorny (1922) not injurious to seedlings of barley.

Beggiatoa lives, according to Ruhland and Hoffmann (1926) for 3 hours in very strongly plasmolysing solution. According to Höfler and Stiegler (1921) cells of Gentiana live for days in 1.0 *n* solution, being at first plasmolysed, but regaining their turgescence after a very short time. They do not believe, however, that the protoplasm is still normal in such a strong solution.

As is seen later on with my own investigation (p. 354 et seq.), cells may apparently be absolutely healthy in a urea solution, whilst they are in reality already so damaged that, when placed in water, they die after some time. It is therefore necessary to be cautious about concluding that urea is not poisonous.

Finally a few publications dealing with *the connection between permeability and age of the cells* must be mentioned. Scheitterer and Weber (1930) and Weber (1931, *b*) found that stomata guard cells of Ranunculus ficaria and Vicia faba were only very slightly permeable for urea when very young, but very much so, on the other hand, when older. With very old, faded, yellow leaves, on the other hand, the permeability again diminished (Weber '31, *b*).

A very curious phenomenon, which is not without interest

in connection with some of my experiments (p. 349), was also found by Weber (1931, *a*). In filaments of Spirogyra some cells were found to be impermeable for urea; in hypertonic solution they remain for a long time plasmolysed and alive. Other cells from the same filaments, on the other hand, are highly permeable (plasmolysis is not even reached in hypertonic solution), and quickly die in urea solution, usually after a few minutes only. The phenomenon mentioned is not due to varying osmotic pressure, as all the cells showed the same degree of plasmolysis in saccharose of 15 %. Weber thinks that there is some reason to suppose that the *younger* cells are permeable for urea; there are, however, exceptions, so that he does not feel justified in coming definitely to this conclusion. We have, therefore, and this is a very remarkable fact, two protoplasmically distinguishable kinds of cells to deal with.

II. My Investigations.

The investigation, which for the rest was arranged exactly in the same way as with the sugar cultures — here, too, the solution was renewed every 3 days and dead cells were removed — were made from July 17th, 1929, to September 5th, 1929, in solutions of 0.05, 0.10, 0.20, and 0.30 *n*, from a standard solution of 1.00 *n*. During the determinations the value of the Nitella cells in water was 0.29 *n* until August 27th, and after that date 0.27 *n* (see table 15*b* p. 293); after August 27th, however, the value of very few cells was determined.

Three series of experiments were made.

1. The change in osmotic value in the 4 solutions mentioned was determined after various times. Only large, fresh green cells were used, which were entirely or almost entirely full-grown.
2. The changes which took place in the above-mentioned fine, green cells in 0.30 *n* urea, were compared with those

which occurred in equally large, full-grown cells, which however were older, which is to be seen by their darker appearance, brittle cellwall, coating of small Algae, and deposit.

3. Cells which had been for a certain time in urea were then kept for varying periods in water. The osmotic value was then determined. From these determinations a conclusion can be arrived at, as is also partly the case with those of the first series, as to the poisonousness of the various solutions.
4. Finally the sap of several cells was chemically tested with a view to determining the presence of urea.

1. *Changes in Osmotic Value in Solutions of Different Concentrations.*

The following table shows the results. Under „Surplus Value" is found the difference between the osmotic value of the cell and that of the urea solution in which it was lying. The further arrangement of the table speaks for itself. The graphic representation, fig. 5, on which the surplus values mentioned are shown belongs to this table.

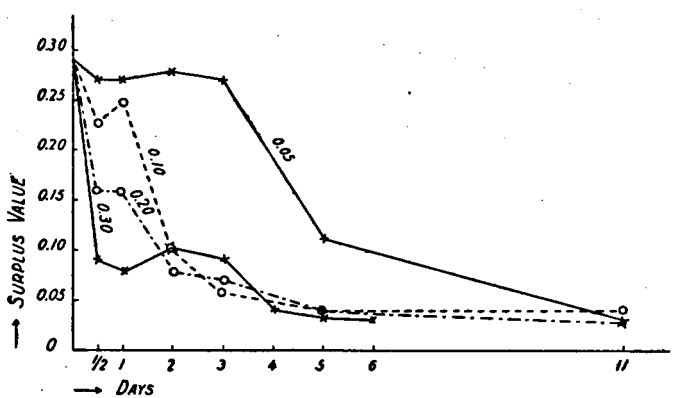

Fig. 5.
Osmotic value of cells grown in urea.

TABLE 36

	After	Osm. values in 0.05 *n*	Average	Surplus-value	Osm. values in 0.10 *n*	Average	Surplus-value	Osm. values in 0.20 *n*	Average	Surplus-value	Osm. values in 0.30 *n*	Average	Surplus-value
Osmot. value in water 0.29 *n*	3½ hours										0.35, 0.35, 0.35	0.35	0.05
	6 „										0.37, 0.38, 0.37	0.37	0.07
	13 „	0.31, 0.32, 0.32	0.32	0.27	0.33, 0.33, 0.33	0.33	0.23	0.35, 0.36, 0.36	0.36	0.16	0.38, 0.39, 0.39, 0.40	0.39	0.09
	1 day	0.33, 0.33, 0.29 0.30, 0.32	0.32	0.27	0.34, 0.36, 0.36, 0.32	0.35	0.25	0.37, 0.33, 0.36, 0.38	0.36	0.16	0.38, 0.38, 0.38, 0.37, 0.37	0.38	0.08
	2 days	0.32, 0.33	0.33	0.28	0.19, 0.19, 0.21	0.20	0.10	0.29, 0.28, 0.26	0.28	0.08	0.40, 0.60, 0.39, 0.40, 0.61, 0.39, 0.40, 0.39	0.40	0.10
	3 „	0.32	0.32	0.27	0.15, 0.16, 0.16	0.16	0.06	0.26, 0.27	0.27	0.07	0.40, 0.38, 0.37, 0.39	0.39	0.09
	4 „										0.33, 0.35	0.34	0.04
Val. in water 0.27 *n*	5 „	0.17, 0.15	0.16	0.11	0.13, 0.12, 0.15, 0.14	0.14	0.04	0.26, 0.23, 0.23, 0.24	0.24	0.04	0.33, 0.32	0.33	0.03
	6 „										0.33	0.33	0.03
	11 „	0.08	0.08	0.03	0.14, 0.13	0.14	0.04	0.23	0.23	0.03			

Table 36 shows, that *in 0.05 n* urea, the value rises very rapidly to 0.32 *n* (8.7 atmospheres), which value after 3 days still is 0.32 *n*; as the water-value at that time was 0.29 *n*, a rise of 0.03 *n* (0.8 atm.) has therefore taken place; after 5 days the value has fallen a very great deal, after 11 days, and probably earlier, it is only 0.03 higher than that of the environment.

In *0.10 n* the osmotic value rises to 0.35 (9.6 atm.), so that the adaptation is nothing like so complete, the rise being only 0.06 *n* or 1.6 atm.; the value then falls in this case also far below the water-value, finally approaching that of the environment (0.10).

In *0.20 n* the value again rises rapidly to 0.36 *n* (9.9 atm.), a rise, that is, of 0.07 *n* (1.9 atm.) with respect to the water-value; a fall then occurs here also.

In *0.30 n* we find a rise to 0.40 *n* (11.1 atm.), (an increase of 0.11 *n* or 2.9 atm.), followed by a fall to approximately that of the environment.

In all these concentrations there is therefore an increase, which increase, however, is far from proportional, in the 4 solutions, to the increase in the concentrations of these solutions (0.05—0.10—0.20—0.30 *n*). The later fall of the osmotic value of the cell-sap is evidently due to the protoplasm becoming permeable for the substances present in the vacuole. Finally all the cells died, so urea has a poisonous action on Nitella, even in 0.05 *n* solution. The graph (fig. 5) shows plainly how long the surplus value subsists in the 4 solutions. The cells in 0.05 *n* stood it the longest (3 days), the curves which indicate the surplus value in the three other solutions „rush" downwards after one day only and approach practically as one line from the surplus value 0.08 to 0.10 *n* asymptotically to the value of the solution in which the cells are lying. Too much importance must not be attached to small differences indicated by the lines from the points which show the values after 2 days in view of the compar-

atively small number of determinations. The curve of 0.10 runs more steeply downward than that of 0.20, and this again more steeply than that of 0.30, since the fall between cell-sap value and concentration of the environment is greatest with the „0.10 cells": 0.25; with the „0.20 cells" 0.16, and smallest with the „0.30 cells": 0.08—0.10. The curve shows clearly that 0.05 *n* urea acts less poisonously than the three stronger solutions. Although it is probable that 0.30 *n* is more poisonous than 0.20 *n* and that this again is more harmful than 0.10 *n*, this cannot be seen by this figure. On this point and with regards to the poisonousness in general I made a number of experiments, which are described below. I must first, however, call attention to a very remarkable phenomenon which occurs with the values I found in 0.30 *n* after 2 days. Amongst these are two cells which had reached a value of 0.60 *n* or 17.7 atm. and had thus adapted themselves completely to the 0.30 *n* solution in which they were lying. I left these two values out of account in calculating the average. I should say that I have never noticed this phenomenon again, not even with the numerous determinations in 0.30 *n* of old, dark-coloured cells (see p. 351, table 37).

Whether they retained these values for a long time I was, of course, unable to determine, as they had to be pricked for the determination, in order to obtain the cell-sap. The cause is probably an individual difference in permeability of the cells for urea and a different degree of resistance to the poisonous action of this substance. That cells from one filament of Spirogyra may display individual differences in behaviour was noticed, though in a different connection, by Weber also (1931, *a*) (see p. 345).

It will have been noticed that the values of Nitella cells cultivated in urea display a greater variability than is the case in water and in sugar; this is to be accounted for by the fact that not all the cells investigated, of course, were

equally large, nor, consequently, the ratio cell-surface to cell-content, which is of importance in the case of an increase in value due to permeability, either. It is therefore necessary, still more than is the case with cells cultivated in other solutions, to make as many determinations as possible. Great caution, however, is advisable with regard to the results of determinations made with mixed cell-sap from several cells, in view of the exceptions above-mentioned.

2. *Difference in Osmotic Adaptation in Old and Young Cells.*

At the beginning of August 1929 comparative determinations were made with the fresh-looking, very green cells, the changes in value of which are given in table 36, p. 347 and with cells which had a very brittle cell-wall, were practically black with deposit and overgrown with Algae. Cells of this kind were invariably long ones. The osmotic value in water of both kinds of cells during the experiment was 0.29 *n*. The following table 37 shows first the values found for the old, dark cells, after these the average of them, and then the average values of green cells, which are also to be found in Table 36. The determinations were made exclusively in 0.30 *n* urea. In the last column is shown the standard deviation of the difference between the mean values of the dark and of the green cells, which is equal to $\pm \sqrt{m_1^2 + m_2^2}$, m_1 and m_2 being the standard deviations of the two mean values shown in the two preceding columns. If the difference between these two means is at least 3 times as large as m_{diff}, then the difference found is actually a real one. With the values after 3½, 6 and 13 hours and after 1 day, which are those of most importance, this is the case; the mean values after 2 days, 0.42 and 0.40, must not be regarded as different, nor those after 3 days, either.

From table 37 and fig. 6 we see that the young, green cells increase their osmotic value more rapidly than the older, dark ones. The young Nitella cells reach their maximum

value in as little as 13 hours (0.39 *n* or 10.8 atm.), this remaining constant for several days, after which the value again begins to fall. The value of the older cells rises, it is true, more slowly, but the maximum is higher; after 1 day this is 0.43 *n* (12.1 atm.). Here, too, a fall then occurs, after which, as with the young cells, the value becomes somewhat higher than that of the environment.

TABLE 37

After	Osm. values in old, black cells cultivated in 0.30 *n* urea	Average	Average of the values of green, young cells	m_{diff}
3½ hours	0.33, 0.33, 0.33, 0.33, 0.34	0.33	0.35	0.002
6 „	0.34, 0.34, 0.34, 0.34	0.34	0.37	0.004
13 „	0.35, 0.35, 0.35	0.35	0.39	0.004
1 day	0.40, 0.45, 0.45, 0.42, 0.40, 0.46, 0.40	0.43	0.38	0.010
2 days	0.46, 0.43, 0.41, 0.39, 0.40	0.42	0.40	0.014
3 „	0.36, 0.38, 0.38	0.37	0.39	0.010
4 „	0.33, 0.33, 0.35, 0.34	0.34	0.34	
5 „	0.32, 0.33, 0.32, 0.32	0.32	0.33	
6 „	0.31, 0.32, 0.32, 0.33	0.32	0.33	
7 „	0.33, 0.32, 0.33, 0.32	0.33		
11 „	0.34, 0.32, 0.33	0.33		
17 „	0.34, 0.32, 0.33	0.33		

Old and young cells grown in urea

The matter may be explained in the following way. The protoplasm, and perhaps also the cell-wall of the younger cells is more permeable for urea than that of older cells, hence the more rapid rise in the case of the former. In consequence of this, however, a relatively large quantity of urea penetrates into the cells, which injures the protoplasm, thus causing exosmosis and consequently a lowering of the value (on chemical demonstration of the urea in the cells see below). In the old cells the injury, owing to the comparatively slow penetration, takes place to a slighter

extent, so that the value is able to rise somewhat higher. Finally, after 1 day, the injury to the protoplasm begins here also, as a result of which the fall in the value of these cells also commences.

Although a direct comparison with the experiments with stomata guard cells performed by Scheitterer and Weber (p. 344) is of course not permissible, a difference in permeability between old and young cells is nevertheless found to exist in the case of Nitella. To what extent the protoplasm and (or) the cell-wall play a further part I am unable to say with certainty. I will merely remark en passant that these experiments could never have been made by the plasmolytic method, since it is impossible to observe the beginning of plasmolysis in the case of these dark cells.

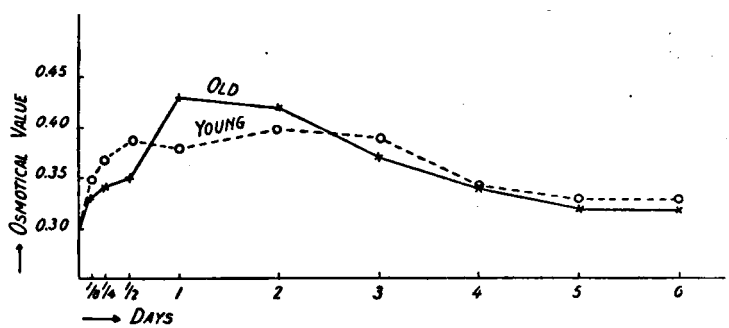

Fig. 6.
Difference between the osmotic adaptation between old and young cells.

3. *Determination of the Poisonousness of Urea in Solutions of 0.05, 0.10, 0.20, and 0.30 n.*

That urea does have a toxic action on Nitella cells has already been seen from the foregoing; at the same time the graph fig. 5 shows that 0.05 *n* acts much less toxically than the other three concentrations. It was, however, impossible to see from the figures and the curve any difference in the action of these three concentrations.

TABLE 38

Concentr. of urea	1 hour in urea. Average	1 hour in urea, then 1½ hour in water	4 hours in urea. Average	4 hours in urea, then 4 hours in water	1 day in urea. Average	1 day in urea, then 3½ hours in water	1 day in urea Average	1 day in urea, then 1 day in water	2 days in urea. Average	2 days in urea, then 11 hours in water
0.05 *n*	0.27	0.25, 0.25, 0.25	0.29	0.28, 0.29, 0.04	0.32	0.27, 0.26	0.32	0.08, 0.06	0.33	0.28, 0.22
0.10 *n*	0.27	0.25, 0.26, 0.21	0.30	0.22	0.35	0.30, 0.09, 0.09	0.35	0.06	0.20	0.05, 0.06, 0,04
0.20 *n*	0.28	0.29, 0.28, 0.26	0.32	0.16, 0.30, 0.28	0.36	0.35, 0.34, 0.34	0.36	0.07, 0.05	0.28	0.06, 0.05, 0.07
0.30 *n*	0.30	0.07, 0.07, 0.22	0.34	0.02, 0.02	0.38	0.08, 0.06, 0.06	0.38	0.05, 0.06	0.40	0.04, 0.04, 0.06

I now first put Nitella cells into various urea solutions for different lengths of time, and then into water for varying periods. The osmotic value was then determined and the condition of the cells examined macroscopically. The investigations were performed on August 27th, 1929, when the *normal value in water was 0.27 n.*

I took from table 36, p. 347, the mean values which the cells had after having been for 1 day and 2 days in urea; I had to find the values after 1 and 4 hours by interpolating between the value in water and that after 13 hours, with the 0.30 *n* culture between the water-value and the value after 3½ and 6 hours. This interpolation was facilitated by the making of a curve (not given here). It was taken into account that, during the observations reported in table 36, the water value was 0.29, whereas this was 0.27 *n* in the case of the values shown in tab. 38.

The table shows the following points.

Cells which in urea were still turgescent, are in many cases found to die when then allowed to remain in water, the osmotic concentration attains a value of little more than 0.

The value determined after the cells had been lying in water was usually either equal to the normal water value 0.27 (or somewhat higher), or very low, which latter case showed that the cells were dying. (With a few exceptions).

This dying being accompanied by exosmosis, seems, therefore, to proceed rapidly, whilst the cells seem to behave differently in this regard. The cells with a high value were invariably pretty turgescent, those with a low value flabby, but otherwise still nice and green, and fresh. Between values like 0.02, 0.05, 0.06, etc., no difference need be made; when the value becomes so low with respect to the environment, the diffusion outwards proceeds very slowly, so that, of course, such differences may very easily arise.

If the osmotic value, and with it the turgescence, after e.g. 3½ hours in water, following on one day in urea, is still

good in the case of many of the cells, the conclusion cannot be drawn with any certainty that these cells are in that case still quite uninjured. This is seen by the table: after 3½ hours the 2 cells tested, which had been in 0.05 *n* urea, still had values of 0.27 and 0.26, one cell out of 0.10 *n* urea had a value of 0.30, and the 3 cells tested out of 0.20 *n* urea values of 0.35, 0.34, and 0.34 *n*, whilst after 1 day in water following on 1 day in urea, the values of all the cells tested approached zero.

The table shows further that 0.30 *n* urea has a more toxic action than the weaker concentrations; for the rest, owing to the small number of observations, no conclusions are to be drawn from this, these determinations giving only a general impression.

In order to see plainly that the toxicity increases with greater concentration of the urea solution, as is to be expected, I made the following further experiment. 40 cells were left for *15 hours in urea* of the 4 concentrations, *after which* they were kept *in main-water for 5 hours*. I then divided them under 3 headings: 1. still perfectly turgescent, 2. flabby, 3. quite dead, the chlorophyll badly deformed. From the figures of table 38 and from what is said on p. 354 we were able to conclude that the flabby cells showed a very low value, approaching zero, the turgescent cells a value differing little from the normal watervalue or even higher.

TABLE 39

Concentr. urea	Still turgescent after 5 hours	Weak after 5 hours	Chloroph. deformed after 5 hours	After 2 days still turgescent	After 4 days still turgescent
0.05 *n*	32	9	0	18	5
0.10 *n*	22	17	1	12	3
0.20 *n*	15	21	4	4	1
0.30 *n*	7	14	19	1	1

Explanation see above.

After 2 and 4 days the number of turgescent cells was counted once again.

Table 39 shows very plainly that the toxicity increases with the degree of concentration.

From all the above experiments it is seen how cautious one must be about concluding that *intact* protoplasm is permeable for urea (I was able to demonstrate chemically that urea actually does permeate; see below). It is very probable that the protoplasm, after being in urea, and before being put into water, was already pathologically modified. As I have already remarked, on p. 344, it is also advisable to be cautious about accepting statements on this point in the literature. The necessity of thoroughly convincing oneself of the undamaged condition of the cells which had been treated with urea was first pointed out in 1928 by Poijärvi, whilst there is everything to be said for the method used by Bärlund (1929) in the case of Rhoeo: he repeatedly plasmolysed, and then deplasmolysed the cells in water; if the cells were still uninjured, the same osmotic value (*Oincip*) would be found in all cases. This was kept up until 24 hours after the first determination. This is not feasible with Nitella, as plasmolysing a few times very quickly injures the Algae.

4. *Microchemical Demonstration of the Presence of Urea in the Cells.*

It is, of course, not quite certain that the increase in the osmotic value is brought about by permeation, however probable this may be. In speaking of the sugar tests I have already pointed out that, if certainty is to be attained on this point, it is necessary to be able to demonstrate the substance in question in the cell-sap. Two microchemical reactions which I applied were the addition to the cell-sap of strong nitric acid or of oxalic acid (see e.g. Rosenthaler (1923, p. 502) and Emich (1926, p. 223).

When urea is present a white, crystalline precipitate is very quickly formed, the reaction being only qualitative. I collected sap from several cells, by pricking them with a glass capillary, put this on an object-glass, and added a drop of nitric acid or oxalic acid. After stirring for some time I found that a white, crystalline precipitate was formed, with nitric acid better than with oxalic acid. The following tests were made.

1. Cell-sap of cells from water showed no reaction.
2. Cells which had been 1 day in 0.10 *n* urea and had reached a value of 0.35 *n*, and 5 cells which had been in 0.20 *n* and had a value of 0.36 *n*, showed a positive reaction, as did also 5 cells which after 2 days in 0.30 *n* urea had a value of 0.40.
3. Cells which had been 1 day in 0.05 *n* urea and had a value of 0.32 *n*, showed only a doubtful cloudiness.

It may therefore be said that the increase in osmotic value is due to permeated urea. That anatonosis has much to do with it seems to me improbable, although, of course, it cannot entirely be ruled out. It may thus be said (on the strength of tab. 36 and of the chemical tests) that the protoplasm of Nitella is permeable for urea, but it must be realised that this protoplasm is probably at the same time already pathologically modified. Experiments which will yield a certain proof of the permeability of normal protoplasm for urea seem to me to be difficult to carry out.

This investigation has shown what a very toxic action urea has on Nitella cells; further, this is the first time, so far as I am aware, that independent cells have ever been cultivated in urea solutions of different concentration and systematic determinations of the osmotic value made with them. My investigation, I believe, renders it doubtful whether the statement made by many (see summary of literature), that healthy protoplasm is permeable for urea, is accurate, apart from the fact that none of them has

made chemical tests. Finally I would remark that none of my tests could possibly have been made with the plasmolytic method, seeing that exosmosis would certainly have occurred in the solution of the plasmolytic (for this happened in the urea solution itself and also afterwards in water), but that *the Barger method is the only practicable one in this case.*

F. CULTURES IN GLYCEROL.

I made very few experiments with glycerol. I will therefore content myself with giving a very concise summary of the literature.

I. Literature.

Here, also, the criterion for permeability is the decrease of the plasmolysis in solutions which at first plasmolyzed. On the strength of this the conclusion is pretty generally reached that most plant-cells are permeable for glycerol. Klebs ('86—'88, '87) first observed plasmolysis in the case of Zygnema; after the decrease the Algae could be further cultivated for months in 10—20 % solution. Hugo de Vries ('88) observed this also with Spirogyra, as did also Overton ('95, p. 196).

With various higher plants deplasmolysis was later found by others, a few plants forming an exception and seeming to be impermeable for glycerol (Fitting, 1920). In some cases glycerol seems quickly to have a toxic action. Nathansohn (1903) states that with Codium tomentosum, after 48 hours in a 10 % solution (which, as a matter of fact, is rather strong), injury occurs.

Von Mayenburg (1901) was able, by means of the acroleine reaction, to demonstrate the presence of glycerol in Aspergillus which had been cultivated in glycerol, which, of course, indicates permeation; so far as I know he is the only investigator who has attempted to show by chemical means the presence of glycerol.

Some writers suppose, on the strength of the fact that a super-regulation of the osmotic value occurs, that not only permeation, but also anatonosis, is the cause of the increase in value.

In connection with the possibility of anatonosis the fact must be taken into account that many plants, very possibly including Nitella, are able to form starch from glycerol.

II. My Investigation.

From September 13th to October 18th, 1929, I cultivated Nitella in 0.10, 0.15 and 0.30 *n* solutions. The water-value during that time was 0.27 *n*. In order to prepare the solutions I started with glycerol manufactured by the „Amsterdamsche Chininefabriek", the specific gravity of which was determined with the aid of Reimann's balance to be 1.2577.

This is a 96.89 % solution (weight percentages), which corresponds to 13.23 *n* (mol p. liter) (for calculation see De Graaf and others (1929, p. 30) as also Fitting (1920, p. 12). By diluting with main-water I prepared the required solutions of this. To make assurance doubly sure, these were checked by Barger's method with boric acid, which yielded the same values.

TABLE 40

After	0.10 *n*	0.15 *n*	0.30 *n*
3½ hours		0.29, 0.30, 0.30	0.31, 0.30, 0.29
6 „		0.29, 0.30, 0.30	0.35, 0.32, 0.32
12½ „		0.30, 0.30, 0.29	dead
1 day	0.26, 0.27	0.30, 0.30, 0.28	
2 days		0.29, 0.31	
4 „	0.24, 0.25, 0.26		
6 „		0.24	
8 „			
9 „	0.26, 0.27		
13 „	0.27, 0.26		
19 „	0.14, 0.15		

We see, therefore, that, after 13 days *in 0.10 n* glycerol, *no increase whatever* has occurred (the water-value is 0.27); the slight rise in 0.15 *n* is perhaps partly due to a diminution in the volume of the cells, which will naturally occur at first in any solution which is at all concentrated; it is also possible that, just as with the cells in 0.30 *n*, glycerol permeated; as the cells in both solutions, especially in 0.30, which plasmolysed somewhat, very rapidly died, it is, however, very probable that the protoplasm was no longer normal. I was struck by the fact that, in the stronger glycerol solutions, the Nitella cells quickly fade and lose their chlorophyll.

It may, however, be deduced from the result of the 0.10 culture that it is none the less very probable that the protoplasm of the Nitella cell is hardly, if at all, permeable for glycerol, which property it would then have in common with several plants investigated by Fitting (see p. 358). By means of various methods I further endeavoured to demonstrate the presence of glycerol microchemically (see Emich [1926 p. 213], but in this I did not succeed; it may be remarked that microchemical determinations of glycerol are extremely difficult to carry out.

I found decrease of plasmolysis in 0.30 *n* solution in hardly any cases; the cells quickly died, as was also the case with cells which had a value of 0.60 *n* in the winter of 1929/'30, and which were plasmolysed in a 0.65 *n* solution.

Fitting (1920 p. 8) remarks, that conclusions as to permeability drawn from the fact of deplasmolysis are only comparable (quite apart from anatonosis W.) if the size of the cells is the same and if the same concentration is worked with. The fact that he sees no decrease, or only a very slow one, of the plasmolysis after several hours, e.g. in the case of Ledenbergia, does not immediately justify the conclusion that there is only a very slight permeability, if

any at all; the size of the cells must be taken into account. Measurements of this were, however, not made with these plants.

It is, of course, not impossible that the protoplasm of Nitella is actually permeable for glycerol, but that this is not yet noticeable after 13 days, owing to the volume of the cells being so large with respect to the surface.

Some support is lent to this view by figures given by Fitting with regard to Tradescantia.

The volume of a cell of the leaf nerve of Tradescantia discolor is, according to Fitting (1917, p. 554, Note 1), 0.0003—0.0007 cc. = about 0.5 cub. mm. The cubic content of a Nitella cell 10 cm. long and 1 mm. thick is 78.5 cub. mm., that is 157 ($= 5.4^3$) times as large. The superficies of this Nitella cell is 315 sq. mm. If we assume the same shape for a Tradescantia cell as for a Nitella cell (which is not quite correct), then the superficies of the Nitella cell is only $5.4^2 = 29$ times as large. (If, of two cells, the content of one is a^3 times as large as that of the other, the surface (which determines the extent of permeation) is only a^2 times as large). According to Fitting (1920, p. 100) the osmotic value is raised in 1 hour from 0.22 to 0.27 *n*, that is 0.05 *n*, when the cell is in a solution which is just plasmolysing. If Nitella were in such a solution it would take $\frac{157}{29} = 5$ times as long to reach an equal value, that is 5 hours. The table shows that the value in the case of Nitella actually has risen about 0.05 *n* after 6 hours in 0.30, 0.03 *n* in 0.15; I do not know, however, to what extent the initial rise is due to a diminution in the size of the cells, whilst it is very likely that the protoplasm is no longer quite intact. As, however, in 0.15 *n* after 6 hours the value no longer shows the slightest increase, permeability is highly improbable. The result in 0.10 *n* also indicates this.

I am therefore convinced that the protoplasm of Nitella is hardly, if at all, permeable for glycerol, whilst no anatonosis can be demonstrated to have occurred either. Further experiments are, however, urgently desirable.

Summary.

Method.

Nitella translucens was cultivated in solutions of various substances, the object of the investigation being to determine the rise in the osmotic value which the cells undergo in these solutions, the cause of this rise, and the factors which influence it.

The well-known methods for the determination of the osmotic value of plants are discussed and the reasons are stated for which they were inapplicable for my purpose. The method used by me was that of Barger, originally intended for the determination of the molecular weight of substances; for the principle on which it is based see p. 238. The degree of accuracy which I considered necessary was about 0.01 n (n = mol p. liter). It is possible to carry out a determination with one cell, as for a determination according to the Barger method a minimum of 1.1 cub. mm. of cell-sap is required, and from one cell of average size about 20 cub. mm. of cell-sap can be obtained.

The variability of the osmotic value of cells cultivated under the same conditions is usually no greater than 8 %; in view of this there is no use in working with greater accuracy than is possible with the Barger method as applied by me. In order to find the value of the cell-sap at a particular moment, I generally took the mean of the value of from 2 to 6 cells, and sometimes also mixed the liquid from several cells and made a determination with this.

All the factors which play a part with the method are

discussed in great detail, as are also biological and chemical applications made by others and improvements introduced by others but which were unnecessary for me.

Physiological investigations.

An annual periodicity of the osmotic value was found to exist; in the summer this is fairly constant, both in 1927, '28, '29, '30, and in 1931 the value was about 0.26 *n*; in the winter the value is higher, but is not the same every year; in January 1929 0.39 *n*, in the winter of '29/'30 0.60 *n*, in the winter of '30/'31 0.33 *n*. These periodical changes were found to occur independently of temperature and light, and they also occur in sugar solutions.

The osmotic concentration of cells cultivated in the dark is little less than those cultivated in the light; low temperatures were found in all the experiments to give a somewhat higher value than high temperatures but to nothing like to such an extent as to account for the periodical increase in the winter.

As the cause of the high winter values I therefore am obliged to assume anatonosis due to unknown autonomous factors, moreover as the periodic rise occurred outward and in the laboratory and either in water or in sugar.

Nitella was cultivated *in saccharose solutions* of 0.10 *n* and 0.20 *n*; a practically complete osmotic adaptation was found to occur.

In *glucose solution* 0.10 *n* the value was increased by 0.07 *n*, in 0.20 solution a complete adaptation came about; the value became 0.46 *n*.

Nitella cannot live in 0.30 *n* solutions.

With winter-cells which had a high value little or no rise was found to take place. It seems, furthermore, that no rise occurs in the dark either in the case of winter-cells or in that of summer-cells.

In the dark Nitella cells can be freed from *starch,* in glucose and saccharose solutions in the dark this is again formed.

With the micro-fermentation method of Van Lutsenburg Maas—Van Iterson sugar tests of the vacuole sap were made; for this method the amounts of sugar fermentable must be between 3.5 mgr. and 0.1 mgr., a drop of 0.010 cc. solution sufficing for one test.

Cells cultivated in water were found to contain no sugar.

My experiments show that the increase in osmotic value of the cells cultivated in 0.10 *saccharose* and *glucose* is entirely due to anatonosis; no sugar whatever was to be demonstrated in the cell-sap. About 13 % of the increase which the Nitella cells undergo in 0.20 *n* saccharose is to be accounted for by sugar, the presence of which could be demonstrated with the above-mentioned method; for the rest it is also an anatonosis. Probably either part of the starch (via sugars) is decomposed into osmotically active substances, or the saccharose (or glucose) is converted into such substances, in the protoplasm, these then permeating through the tonoplast.

In 0.20 glucose, when the osmotic concentration of the cell-sap has reached 0.44 *n,* more than half the rise is to be accounted for by sugar; 10 days later, when the value is 0.47 *n,* almost the entire rise.

In these cells, therefore, anatonosis occurs; as later on, when the osmotic value has reached its maximum, a great deal of sugar can be shown to be present in the cell-sap, it is probable that permeation of the sugar then gets the upper-hand of the anatonosis. It cannot, however, be considered impossible that the starch is then decomposed, which is also an anatonosis.

The experiments with *urea* gave the following result. Nitella was cultivated in solutions of four concentrations;

the smaller the concentration of the solution was, the more complete was the osmotic adaptation. By means of micro-chemical reactions it was possible to demonstrate that this increase was due to permeated urea.

After having reached a maximum value the value then fell again, until it was about the same as that of the environment, so that the urea evidently had a toxic action. With the object of investigating this more closely, cells were put into water, after having been for a long time in urea of different concentrations; at that moment they were evidently still in a healthy condition, but it was found that the osmotic value in water fell after a longer or shorter period and finally approached zero — the cells died.

It is therefore not impossible that, after being in urea, and before being transferred to water, the cells were already no longer healthy. This shows how cautious one must be in assuming the permeability for urea of *intact* protoplasm.

I was able to show that — as was, indeed, to be anticipated — the toxic action of urea on Nitella increases as the concentration of the solution rises.

Comparative determinations were further made of fresh green cells, such as were used for the other experiments, with other cells which were darker, coated with Algae and dirt, and which had a much more brittle cellwall; it was found that the course of the changes in osmotic value in urea varied (table 37).

Cultivated in *glycerol* (0.10 *n*) the osmotic value after 13 days was still equal to the initial value, 0.26 *n* to 0.27 *n*. I have concluded from this that the protoplasm of Nitella is scarcely, if at all, permeable for glycerol.

In 0.15 and 0.30 *n* solutions the cells quickly died.

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