Dr. SILVESTR PRÁT:

Plasmolysis and Permeability II.*)

The formula of calculation of osmotic pressure of plant cells by HÖFLER $G = \frac{V_p}{V_z}$, $O = C \times G$ can be used only for regular cylindrical cells.

V_p — volume of protoplast after the contraction.
V_z — inward volume of the cell.
G — the degree of plasmolysis.
O — the osmotic pressure.
C — the concentration of plasmolyticum.

But a little modification permits the application of this method also for the cells of irregular form, if they only have the parallelogram-form in the plane vertical to the optical plane of the microscope (eventually with arched walls). Fig. I a, b.

We draw the plasmolyzing cells accurately with a drawing-apparatus or we make a microphoto and follow the contractions or dilatations of the protoplast — and thereby the changes of the degree of plasmolysis — by measuring with the planimeter; or we cut out the cells and the protoplasts and compare the weight of the papers.

This modification makes the plasmometric method much more applicable. The cells with a parallelogram intersection, on which the protoplast cannot change its volume in the plane vertical to the plane of observation (invisible changes) but which permit all the changes of protoplast volume to follow in the optical plane of the microscope, are very frequent in plants. They are very significant for the leaves of mosses, for epidermis etc.

Great accuracy is not to be expected from this method of determining the osmotic pressure. Because by this modification the measure or the weight of the plan of protoplast and cell does not exactly correspond with the volumeproportions; therefore also this method cannot be used for exactly measuring the osmotic pressure of the cells, which must be carried out by the old method by de VRIES, by the method of beginning (incipient) plasmolysis.

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*) S. Prát: Plasmolyse und Permeabilität. I. Biochem. Zeitschr. 128. 1922. 557.

But the plasmometric, also modified method, always gives at least the approximate value and it is a great saving of time and material. Several solutions differing slightly in concentration complete the observations with the method of beginning plasmolysis, which otherwise, especially in new plant-objects, needs a very great number of solutions of different concentrations.

A still greater significance has this method for determining the permeability of plasmolytica. In these attempts we do not need absolute value of osmotic pressure, but only relative numbers expressing the change in the degree of plasmolysis. If we see that the volume of protoplast changes in a definite time, then even the method of beginning plasmolysis cannot indicate more correctly the osmotic value of the cell. But if the degree of plasmolysis after the contraction remains constant at least for some time, if therefore the plasmolyticum is very impermeable, the method of beginning plasmolysis indicates eventually more exact values.

And surely this method is very applicable in the experiments which compare the permeability of one tissue for different salts or in general different plasmolytica and in different external conditions, because in the same object the errors are always the same, but the relative numbers can very well be compared with each other.

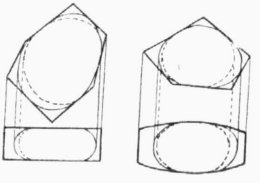

Fig. 1. a, b.

A series of experiments was designed to test the possibility that this modification of the plasmometric method can be used for measuring permeability. The experiments reported in the present Bulletin include studies of *Mnium* and plasmolytica KNO_3, saccharose. Generally *Mnium affine* was employed, but in some case *Mnium hornum* was also used as an indicator.

The plants of *Mnium hornum* after having grown 7 days in drinking water, were rinsed thoroughly in duplicate solutions to prevent dilution of those used in the experiments, and were placed in the desired concentrations of plasmolytica. On the 1. VII. 1920 at 9 h 15 min the young leaves were placed into m KNO_3. From 11 h 45 min till 12 h the cells were drawn (Zeiss-Abbé), and the outlines then accuratelly cut out.

The relation $\frac{\text{weight of the protoplast}}{\text{weight of the cell}} = \frac{V_p}{V_z}$

0·38, 0·40, 0·38, 0·34, 0·44, 0·42, 0·35, 0·36, 0·41 — — — 0·38 m KNO_3

0·40, 0·42, 0·43, 0·40, 0·40, 0·41, 0·37, 0·40, 0·44 — — — 0·41 m KNO_3

9 h 45 min in saccharose. Drawn from 13 h 47 min to 13 h 55 min:

0·44, 0·50, 0·44, 0·54, 0·44, 0·42, 0·40, 0·44, 0·44, 0·43 — — — 0·45 m sacch.

18 h 6 min — 18 h 12 min:

0·48, 0·58, 0·50, 0·50, 0·48, 0·50, 0·48, 0·48, 0·48, 0·52 — — — 0·50 m sacch.

M n i u m a f f i n e. The plants grew in a covered glass-dish in damp air. 3 VII. 1921. 10 h 45 min. The young leaves were plasmolysed with ½ m KNO_3. Drawn 14 h—14 h 5 min. The relations: (24° C)

0·30, 0·28, 0·28, 0·29, 0·28, 0·28, 0·30, 0·28, 0·30, 0·33 — — — 0·29 m KNO_3

17 h 40 min — 17 h 48 min

0·34, 0·32, 0·33, 0·30, 0·32, 0·33, 0·35, 0·35, 0·35, 0·35 — — — 0·33 m KNO_3

In the solution of m/3 KNO_3 in 14 and also in 18 hours the plasmolysis was very irregular, some cells were more or less plasmolysed, some were not plasmolysed; both methods produced the same results, nearly 0·3 m KNO_3; more exact determining of boundary concentration was impossible, as differences of some single cells were pretty considerable. Copeland already in 1896 indicates the difference in one leaf of *Mnium* till 1% KNO_3.

3. VII. 1921. Young leaves *Mnium affine* 9 h 45 min m saccharose. Drawn: 13 h 38 min — 13 h 48 min. $O = C \times G$

0·52, 0·54, 0·52, 0·52, 0·52, 0·52, 0·48, 0·48, 0·54, 0·52 — — — 0·516 m sacchar.

18 h — 18 h 06 min:

0·52, 0·48, 0·52, 0·52, 0·48, 0·50, 0·50, 0·48, 0·50, 0·44 — — — 0·49 m saccar.

In the solution of m/2 cane sugar after some hours the beginnings of plasmolysis were observed, after 24 hours there was no change (the maiority of cells were not strongly plasmolysed).

⅔ m KNO_3 10 h 45 min. Drawn from 14 h 14 min to 14 h 18 min:

0·42, 0·42, 0·40, 0·40, 0·42, 0·40, 0·48, 0·32 — — — 0·407 m KNO_3.

Another leaf:

0·42, 0·42, 0·48, 0·44, 0·44, 0·44, 0·46, 0·38, 0·50, 0·46 — — — 0·44 m KNO_3

17 h 52 min — 17 h 59 min:

0·44, 0·38, 0·42, 0·44, 0·38, 0·42, 0·38, 0·40, 0·42, 0·44 — — — 0·41 m KNO_3

7. VII. 1920. Young leaves of *Mnium affine* were placed in the solutions KNO_3 ⅓ m, ½ m, ⅔ m (14 h 45 min) cane sugar ⅓ m, ½ m (14 h 55 min).

The results:

⅓ m KNO_3 16 h 58 min — 17 h 05 min:

0·27, 0·27, 0·26, 0·26, 0·27, 0·26, 0·26, 0·26, 0·25, 0·27 — — — 0·26 m KNO_3

½ m KNO_3 16 h 50 min — 16 h 55 min:

0·27, 0·29, 0·29, 0·28, 0·28, 0·28, 0·35, 0·27, 0·29, 0·30 (— — — 0·29 m KNO_3)

without abnorm. 0·35 — — — 0·28 m KNO_3

After 20 hours the cells in ⅓ m KNO_3 were deplasmolysed, in the solution of ½ m KNO_3 only rare weak plasmolysis was observed.

⅔ m KNO_3 16 h 40 min — 16 h 48 min;

0·38, 0·38, 0·36, 0·36, 0·36, 0·32, 0·34, 0·32, 0·28, 0·34 — — — 0·34 m KNO_3

⅔ m KNO_3 8. VII. 9 h 15 min — 9 h 25 min:

0·50, 0·44, 0·42, 0·40, 0·52, 0·44, 0·40, 0·48, 0·50, 0·52 — — — 0·46 m KNO_3

In these experiments very distinct permeability can be observed. In the latter case beside this it was ascertained that considerable differences exist also between the neighbouring cells. The increase of the value of osmotic pressure in higher concentrations of KNO_3 respondes to „Protoplasmacorrectur" by Höfler.

⅓ m saccharose — no plasmolysis.

½ m sacchar. 17 h 10 min — 17 h 15 min.

0·31, 0·33, 0·30, 0·31, 0·31, 0·29, 0·29, 0·29, 0·31, 0·31 — — — 0·305 m sacchar.

0·31, 0·34, 0·33, 0·30 — — — 0·32 m sacchar.

8. VII. 9 h 36 min — 9 h 43 min.

0·40, 0·37, 0·34, 0·37, 0·31, 0·38, 0·35, 0·34, 0·36, 0·35 — — — 0·347 m sacchar.

On the 8. VII. 12 h 50 min a leaf of *Mnium affine* was placed in the solution of ⅔ m KNO_3, at 13 h 30 min the preparation with vaseline was closed. The data of observation and measuring in fixed time-intervals which were obtained are summarised in the table. In the first hour of observation (till two hours from the commencement of plasmolysis) the degree of plasmolysis was constant, but then it became rapid and later slow increase was again observed. In closing this rewiew a diagram is given which presents in a graphic form the cardinal numerical relations.

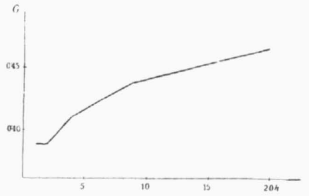

Fig. 2.
Mnium affine, ⅔ m KNO_3.

8. VII. 1920.	Time:	Hours:	Osmotic pressure:
A	13 h 50 min — 13 h 55 min	1	0·387 m KNO_3
B	14 h 55 min — 15 h 01 min	2	0·387 m KNO_3
C	16 h 50 min — 16 h 55 min	4	0·410 m KNO_3
D	19 h 15 min — 19 h 20 min	6½	0·425 m KNO_3
E	21 h 36 min — 21 h 40 min	8¾	0·437 m KNO_3
9. VII. 1920.			
F	9 h 00 min — 9 h 06 min	20	0·465 m KNO_3

In view of the results above presented, it appears that *Mnium affine* just begins to plasmolyse in a cane sugar solution having a concentration of 0·5 mol., in a KNO_3 solution of 0·3 mol. The penetration of KNO_3 is supposed to be largely responsible for the higher value obtained with this salt. Also in the solution of saccharose the dilatation of protoplast was observed, but it was very slow.

Already in the year 1915/16 I repeated the experiments of Szücs on the effect of H_2O_2 on the permeability of Spirogyra. The preparation Perhydrol Merck and a medical solution of hydroperoxyd were used after the dilution. Tufts of Spirogyra filaments placed in the solution always emitted bubbles of oxygen (katalase). The difference in the action of the original solution (weak sour) and the solution after the neutralisation was very strong and the following table includes some of these results:

	$1^0/_0$ H_2O_2	$1^0/_0$ H_2O_2 + $CaCO_3$
10 min.	—	numerous little bubbles
20 „	little bubbles	very „ „ „
30 „	numerous „ „	„ „ large „
40 „	„ „ „	large bubbles ascend to the surface
50 „		
70 „	large bubbles	the bubbles burst at the surface.
120 „	„ „ „ ascend to the surface.	

A comparison of *Spirogyra* and *Zygnema* reveals the fact, that *Zygnema* is far more resistant to H_2O_2 and equally to other substances. The bubbles produced by *Zygnema* in H_2O_2 were never so large as by *Spirogyra* and the developement was far slower.

The observation of Szücs, that H_2O_2 accelerates the penetration of $FeSO_4$ in the cells of *Spirogyra*, was confirmed. The following includes only one of these experiments as an example: 5 ccm of the 2·75% solution H_2O_2 of very weak sour reaction were diluted after the neutralisation with $NaHCO_3$ with 45ccm water. Tufts of *Spirogyra* were placed in this solution and left for 5min. After being rinsed thoroughly 10min. in water changed three times, they were placed in the solution of $FeSO_4$ (Merck $1^0/_{00}$, $^1/_2{}^0/_{00}$, $^1/_4{}^0/_{00}$, water doubly destilled). At the same time a second series (*Spirogyra* without H_2O_2 influence) was observed for the purpose of control. After one hour this was the result of the observations: in normal filaments of *Spirogyra* only a few damaged cells were dyed blue, healthy cells were not at all changed.

It the cells after H_2O_2 action tannin was precipitated, in dead cells the nucleus was also blue. After 4 hours the difference was still clearer.

Zygnema was also examined and gave the same results. Eosin penetrated more rapidly into the cells of *Spirogyra* after the action of H_2O_2, but the red cells (nucleus and protoplasma) were always dead. The measuring of permeability by the plasmometric method was almost impossible. The filaments of *Spirogyra* after the action of H_2O_2 — also of short duration — were much injured by the plasmolyzing solutions, the plasmolysis was very iregular and the cells died very soon; the cells which remained alive somewhast longer, had very irregular menisci, were often square-built and therefore the measuring was very incorrect. The correct investigation of the osmotic pressure with the method by de Vries was also impossible as the plasmolysis was very irregular.

In determining whether any substances (narcotica) may produce an increase of permeability to colouring matters the action of aniline was very interesting. The aniline used in the experiments was four times destilled and perfectly colourless.

The filaments of *Spirogyra* placed in the solutions of methylene blue or neutral red with 0·05—0·1% aniline, were always coloured much quicker than in an aqueous solution alone.

This effect was remarkable only if aniline was added to the solution of colouring matter. If *Spirogyra* was first subjected to the action of aniline in 0·01—0·15% aqueous solution (½—1 hour) and then the filaments were placed in the colouringmatter solution, no differences on intact cells

were visible. Beside this, aniline makes the celerity of tinction greater only in colouring matters which penetrate normally and it was impossible to obtain the vital tinction in non penetrating colours (aniline blue, eosin, indigocarmin).

Methylene blue in aniline solutions became green and latter a brown sediment was formed. The chemical reaction of aniline and methylene blue was also not excluded. But also penetration of $NaHNH_4PO_4$ and $FeSO_4$ became greater with the influence of aniline. In the more concentrated solutions of $FeSO_4$ aniline precipitated $Fe(OH)_3$; this often sedimentated on the sheath of *Spirogyra*.

Spirogyra lutetiana. 5. V. 1916.

	$NaHNH_4PO_4$		$NaHNH_4OP_4 + 0{\cdot}2\%$ anilin	
	m/20	m/40	m/20	m/40
1 hour	white precipitate	—	greater quantity of white precipitate	—
2 hours	„ „	—	„ „ „ „ „	—
6—7 hours	„ „	—	„ „ „ „ „	precipit.

The solution of $FeCl_3$ did not only colour the sediment, but blue was diffused through the whole cells. In the 10% solution of $K_2Cr_2O_7$ the grains of sediment were coloured brown.

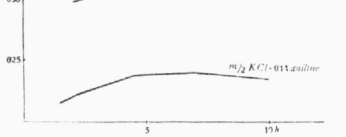

Fig. 3. Spirogyra.

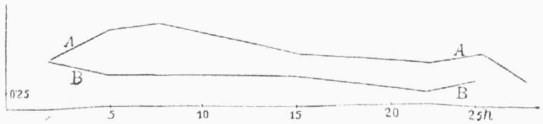

Fig. 4. Spirogyra. A. m/2 NaCl. B. 22 deys in a $0{\cdot}1\%$ solut. of aniline, m/2 NaCl.

The best concentration of aniline must be found in the experiments with $NaHNH_4PO_4$; otherwise the results are not clear. In the case mentioned in the table, m/40 solution made no precipitation in intact cells; but in other species of *Spirogyra* the precipitation was so quick, that the effect of aniline was not remerkable; m/300 solution makes in normal cells no precipitation after 20 minutes; but in the same concentration with anilin distinct, gray, gross enough precipitate was formed. After 40 min. the grains also in control series were visible, but in the solution with aniline the quantity was greater.

Very distinct was the influence of anilin upon the penetration and blue precipitation of $FeSO_4$. A pure aqueous solution of $FeSO_4$ (0·06—0·125%) did not visibly penetrate into the cells of *Spirogyra* either living or dead. But in a solution o the same concentration with 0·1—0·125 aniline there were also often many intact cells with a great quantity of blue precipitate (after 20 min.). In the dead cells there was always very gross sediment. Some coloured cells it was possible to plasmolyse. After 60 min. the quantity of precipitate was still greater; but the plasmolysis was very irregular or impossible.

In diluted solutions of aniline *Spirogyra* lived very long without visible changes. Plasmolysis in the cells after the action of aniline was often better than in normal cells. Menisci were always very regular. No case was ever observed in the best cultures of 8 cells indicating the same degree of plasmolysis (G = 0·430, exp. no. 4.) as in an experiment with 0·1% aniline. Therefore the study of the action of aniline on plasmolysis was very possible. But the plasmometric method never indicated an increase of permeability, however little, caused by the action of aniline. In some cases lower osmotic pressure in m/2 KCl+0·1% aniline was observed, but mostly the osmotic value of the cells with aniline was higher and the increase of the degree of plasmolysis was always slower. 10—14 days after *Spirogyra* had been placed in 0·1% solution of aniline the decrease of the degree of plasmolysis was apparent. The following table includes some of the most significant of these results:

Spirogyra, at K r č (near Prague)

½ m KCl

Hours:	G Maximum	G Minimum	G	G × C = O
2 h —	0·62	0·57	0·594	0·297 m KCl
4 h 15 min.	67	57	0·624	0·312
6 h 45 min.	70	60	0·645	0·322
9 h 50 min.	75	63	0·677	0·338
12 h 50 min.	72	65	0·684	0·342
Idem, ½ m KCl + 0·1% aniline:				
1 h 25 min.	0·43	0·43	0·430	0·215 m KCl
2 h 10 min.	45	43	0·444	0·222
4 h 30 min.	56	45	0·477	0·238
7 h —	50	46	0·480	0·240
10 h —			0·47	0·235

Spirogyra, at K r č

½ m NaCl 12 cells.

Hours:	G Maximum	G Minimum	G	G × C = O
1 h 35 min.	0·70	0·45	0·568	0·284 m NaCl
5 h —	72	52	0·623	0·311
7 h 45 min.	72	52	0·634	0·317
15 h 15 min.	68	47	0·562	0·281
22 h 35 min.	63	49	0·537	0·268
25 h 25 min.	69	48	0·564	0·282
27 h 35 min.	63	46	0·512	0·256
Idem, *Spirogyra* 22 days in a 0·1% solut. of aniline, ½ m NaCl:				
1 h 35 min.	0·66	0.48	0·562	0·281 m NaCl
4 h 50 min.	68	46	0·529	0·264
7 h 25 min.	64	45	0·525	0·262
15 h —	63	43	0·515	0·258
22 h 20 min.	57	40	0·468	0·234
25 h —	55	43	0·500	0·250

Summary.

1. Hofler's plasmometric method of determining osmotic pressure and permeability is applicable also in cells of irregular form *(Mnium)*.

2. Diluted solutions of aniline cause an accelerated penetration of methylene blue, neutral red, $FeSO_4$ and $NaHNH_4PO_4$ in the cells of *Spirogyra*.

The plasmometric method indicates a decrease of permeability of *Spirogyra* for KCl and NaCl after the action of diluted aniline-solutions.

Literature.

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Ústav pro fysiologii rostlin Karlovy university v Praze.