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COMPARISON STUDIES OF THE PERMEABILITY OF LOWER PLANTS

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I - INTRODUCTION

Last decade, when systematic studies of the permeability of plant cells for numerous different aneleotrolytes were begun, only such objects were initially used which seemed apt to give the most accurate results when studied according to a certain method. After comprehensive studies (RUHLAND & C. HOFFMANN 1925; BARLUND 1929; SCHONFELDER 1931; HOFLER 1932-34 and 1936; COLLANDER & BARLUND 1933; v. HOFE 1933; WAHRY 1936; C. HOFFMANN 1936) thus creating a firm foundation for the continued study of the subject, the work program was expanded to include a larger number of objects but fewer compounds. In these tests as well, the choice of objects was largely determined by how well they were suited to be studied according to a certain, predetermined method. This procedure, however, left the permeability properties of some plant groups as well as of some physiological-anatomical cell types totally unclarified due to the methodical difficulties connected with their examination. Under these circumstances, it seemed desirable that answers be found in particular as to the permeability properties of the representatives of just these groups and that these objects thereby be drawn within the scope of the comparative permeability studies.

The work program described hereafter is based on just this principle. It was dictated mainly by the desire to extend the investigation to such systematic groups (Bacteria, Cyanophyceae, Rhodophyceae, etc.) or physiological-anatomical cell groups (embryonic cells) whose permeability properties had so far been investigated in part only or not at all. Under these circumstances, it was not possible, of course, to predetermine the investigation method, and it is for this reason that almost every object was studied according to a different osmotic method, namely that which

seemed most appropriate for whatever object was under study. Although this procedure involved a certain increased expenditure of work, it nevertheless had the great advantage that one and the same person, while working with such diversified methods, was in a position to be a critical judge of their applicability and, at the same time, develop them further, thus contributing to the methodology of the study of permeability.

Most of my test objects are hydrophytes. Consequently, they were practically in their natural element during the tests. Sectioning was necessary only with the roots of Lupinus. The effect of wound irritation on the permeability properties of the plasm should therefore be very limited in these tests.

Since my study is a direct continuation of the work of MARKLUND (1936), I was mainly investigating the permeation capacity of the same anelectrolytes he had used in his study. MARKLUND, in setting up his work program, chose from among the number studied by BARLUND (1929) 12-15 anelectrolytes which were generally harmless to the test object, or not too toxic, but which at the same time were sufficiently water-soluble and whose chemical composition, lipin solubility, and particle size differed as widely as possible.

The following 12 anelectrolytes formed the matrix of the compounds used: saccarose, erythrite, malonamide, glycerine, urotropin, urea, methyl urea, ethylene glycol, acetic amide, propionamide, antipyrin, and trimethylcitrate. None of the rapidly permeating compounds could be tested in the experiments with bacteria. To make up for this, I used instead a sizeable number of slowly permeating substances (raffinose, mannitol, glucose, rhamnose, glycocchol, and methylglucoside).

The substances used -- with the exception of raffinose, rhamnose, and glycocchol -- were identical to those used by BARLUND (chapter 1, p. 16-). The raffinose, rhamnose, and glycocchol were the purest products of Schering-Kahlbaum.

The acetamide, propionamide, butyramide, and diethylurea as well as the methylglucoside were washed with ether before use. The dimethylcitrate solution was neutralized with finely pulverized $CaCO_3$.

The abbreviations of the chemicals used are the same, here as well as hereinafter, as in COLLANDER & BARLUND (1933, p. 16).

II - BACTERIA

1. PREVIOUS KNOWLEDGE CONCERNING THE PERMEABILITY OF BACTERIA

The only bacterium whose permeability properties have so far been thoroughly studied is the peculiar sulfur bacterium Beggiatoa mirabilis. RUHLAND & HOFFMANN (1925) as well as SCHONFELDER (1931) used the breaking method to determine the limit concentrations of numerous electrolytes and of even more numerous anelectrolytes for Beggiatoa. The results showed definitely that the permeation capacity of the compounds studied is determined first of all by the particle size while, in this case, the lipin solubility is of secondary importance. Beggiatoa has thus become the classic example of the ultra filter theory.

At the end of their paper, RUHLAND & HOFFMANN (col. 1, p. 80) submit the following (diminishing) series: Beggiatoa mirabilis > kidney epithel cells > bacteria (?) > ordinary plant cells. Thus, it seems that the above-named authors consider the protoplasts of bacteria more permeable than those of other plants, although with reservations as their question mark seems to indicate. Indeed, very little is known, even today, about the permeability of "ordinary" bacteria, most probably because the minute size of the bacteria cells makes it extremely difficult to measure their permeability.

FISCHER (1895; 1903) plasmolytically studied the permeation of some anelectrolytes into the cells of numerous kinds of bacteria. Of the various plasmolytica included in my work program, antipyrin, urea, and glycerine did not effect any plasmolysis whatsoever in the bacteria cells. In their behavior to cane sugar solutions for example, two groups of bacteria emerged: 1. plasmolyzable bacteria, comprising most gram-negative bacteria, and 2. non-plasmolyzable bacteria, comprising most gram-positive bacteria. In the bacteria cells of the former group, an acute plasmolysis, induced by a 15% (0.440 GM) cane sugar solution, reversed within 1-2 hours (FISCHER 1895, p. 160), sometimes even considerably faster, which is very rapid in comparison to the plasmolysis of other protoplasts.

HYLKEMA (1916) studied the permeability conditions of bacterium coli cells according to the sediment method. "Here the permeability condition is judged to be as it is when the bacilli have been not more than one half hour in the solution in question." (p. 78). Since the solutions of urea, glycerine, and mannitol used (s. col. 1, Table V, p. 152-) did not cause any "inkrimping" of the B. coli cells after such a period of time, HYLKEMA (col. 1, p. 79) considers the cells as completely permeable for the above-mentioned substances.

HILL (1929), on the other hand, experimented with photogenic Bacillus Fischeri cells, whereby he used as indicator of permeation the time within which the glowing in variably strong solutions disappears.

In a 1.0-mol. glycerine solution, such time was only 20% longer, in a 1.0-mol. urea solution, however, three times as long (41 seconds) as in pure water (15 seconds). Thus, both substances penetrate extremely rapidly into the bacteria cells, but "urea did not penetrate as readily as ..... glycerine" (col. 1, p. 868).

Due to the fact that the process used as indicator of permeation took place extremely rapidly in all of the above tests, the bacteria protoplasts in general were considered especially permeable with respect to all of the aneleotrolytes tested and even with respect to substances such as mannitol and cane sugar whose penetration is otherwise hardly noticeable. In respect to permeability conditions, the bacteria seemed to be in an almost special category, and a closer investigation therefore promised interesting results.

2. EXPERIMENTS WITH GRAM-NEGATIVE BACTERIA

The Test Objects and their Culture. Forms of Plasmolysis. - For these tests, I obtained several pure cultures of the Bacterium coli group from the Bacteriological-Serological Institute of the University of Helsinki. The first decision was to determine which of these bacteria were most suitable for use as test objects. The fact that I had chosen the so-called limit plasmolysis process as my test method required of the test object that the plasmolysis be clearly perceptible. It was found that of the accessible bacteria, Bacterium paracoli "4a8", B. coli "Randen", and B. aerogenes "Nordlund" (s. C. & MAJA NYBERG 1933, p. 4-) met this requirement excellently.

The bacteria culture was made on agar-agar (1.5% agar-agar, 0.5% meat extract, 1% pepton, and 1% cane sugar; (s. FISCHER 1903, p. 24). The preparation of the culture media and the culture of the bacteria took place in the usual manner. The cultures were allowed to develop during 14-16 hours in the warming cabinet at 35° C. A fairly homogeneous bacteria material was obtained in which the individual cells attained greater length than in older cultures.

In these rod-shaped bacteria, the plasmolysis manifested itself in very regular, convex form. In weakly plasmolyzing solutions, the cell content in the shorter rods retracted from one cell end (drawing 1a), in the longer rods it accumulated at both cell ends (drawing 1b). With stronger plasmolysis, the cell content in the shorter rods constricted itself into two parts, in the longer rods into several parts (drawings 1c and d, respectively) (comp. FISCHER 1895 and 1903; RAICHEL 1928), whereby the length of the plasma clots as well as of the intermittent empty spaces varied.

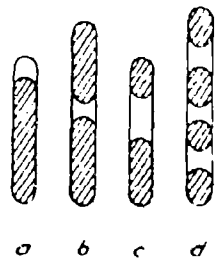

Figure 1. Plasmolyzed B. paracoli cells. Detailed explanation included in text.

In the actual permeability tests, it was not possible to follow these detailed forms of plasmolysis but the attention had to be directed above all to the general picture provided by the plasmolyzed and unplasmolyzed (or deplasmolyzed) bacteria. The cell contents of the plasmolyzed bacteria appeared dark, sometimes almost black, due to the refraction of light, the empty spaces between them light, whereas the unplasmolyzed bacteria showed an even, gray color.

In order to be able, later on, to distinguish beyond question between the weakly plasmolyzed bacteria and those cells which had not undergone any plasmolysis at all, I took under the microscope, alternately, bacteria from water and from variably strong cane sugar solutions. Slowly, (in about two weeks) the eye became experienced in making a reliable distinction between the plasmolyzed and unplasmolyzed cells and, simultaneously, in determining the degree of plasmolysis. I made the first evaluations of the degree of plasmolysis in the cells in the field of vision with the help of a second person, in such a manner that both, alternately, appraised the degree of plasmolysis in the entire field of vision and then compared the results obtained. In this way, the eye became conditioned to greater objectivity in appraising the degree of plasmolysis.

The Test Methodology. - After numerous preliminary experiments, I finally arrived at the following sequence in carrying out the actual experiments:

Shortly before the experiments were started, the required stock solutions were prepared in normal volume, in measuring flasks. In all cases, ordinary tap water was used as solvent (s. BARLUND 1929, p. 18). The individual series of solutions were obtained by diluting the stock solution; the difference in concentration between the various links of one series usually was either 0.02 GM or 0.05 GM. The test solutions were kept in small, stoppered, cylindrical glass containers. The solution series were prepared fresh each day.

During the tests, I used both forms of the limit plasmolytical method, i.e. the "total" pressure method as well as the partial pressure method with, however, some modifications made necessary in view of the special properties of the test object. In conducting the tests, I was forced to use one single solution only at a time before proceeding to the next one because the plasmolysis in the bacteria cells reversed rapidly.

In the case of short experimentation time, I first mounted a slide on the stage of the microscope. By means of a platinum-wire loop I removed a small portion of the bacteria culture from the test tube and mixed it as evenly as possible with a drop of the solution to be examined, which had previously been placed on the slide, until the mixture turned gray. The stop watch was started simultaneously. After rapid mounting of a cover glass, the entire field of vision was then studied at oil immersion. The first observation usually took place within 20-30 seconds, in rarer cases after 30-45 seconds. Close observation of the process was continued until the degree of plasmolysis could be evaluated ocularly at 50%. To be on the safe side, deplasmolysis was also observed after another 1-2 minutes had elapsed.

In longer experiments, I withdrew 1 - 0.2 cubic centimeter of the solution to be tested into a small tube equipped with a glass stopper. Into this amount the bacteria was then homogeneously dispersed by agitation, and the first observation was made after at least three minutes had elapsed. The next observation followed immediately thereafter, in intervals of 1-2 minutes until the 50% degree of plasmolysis was reached again, after which, for control purposes, the further course of plasmolysis was still observed. (For each observation, a new drop of the bacteria suspension was put onto the slide.) I then proceeded to the next solution, either stronger or weaker depending on which end of the series I had begun.

In the tests conducted with the partial pressure method, the individual links of the solution series contained 0.6 GM of the substance to be tested as well as cane sugar in graded concentrations ranging from 0.02 GM to 0.2 GM, in declining succession (osmotic value of the bacteria cells 0.18 GM, or 0.19 GM cane sugar). The number of solutions was dependent on the plasmolyzing effect of the compound to be tested. During the tests, the first observation had to take place as soon as possible since the clearly plasmolyzing effect of the compound solution lasted an extremely short time only. The observation technique, therefore, had to be perfected so as to allow rapid observation of the plasmolysis process. My method, therefore, was as follows:

As before, the slide was put onto the stage of the microscope. Now, however, I fastened the cover glass to the immersion objective itself by means of cedar oil. Again (as was done previously), some of the bacteria were stirred rapidly into a drop of the solution to be tested, which had already been put onto the slide, and the microscope was simultaneously

focused on the test object. This manipulation took generally about eight seconds. Observation, however, could only commence after 10-12 seconds had elapsed since the mounting of the cover glass caused violent motion in the solution, which made observation difficult or ruled it out completely.

The determination of the time necessary to reach limit plasmolysis was always made at least twice with the same solution; during the second determination I kept the stop watch face down on the table to make certain that the result was not influenced by subjective expectations. For the same purpose, I sometimes quite arbitrarily interchanged the individual links of the solution series (I had labeled the test containers at the bottom). Furthermore, not only at the beginning of my work but also between the various tests, I observed the bacteria in pure water to obtain a definite picture of the unplasmolyzed bacteria cells. This provided the opportunity for testing the results which, indeed, did not show wide fluctuations. - I always determined the cane sugar value of the bacteria before and after the tests. The difference was always less than 0.01 GM.

Like BARLUND (1929) and MARKLUND (1936), I organized the tests in such a way that the plasmolyzing effect of at least two compounds could be simultaneously determined in two similarly constituted bacteria cells which had been taken from the same point on the surface of the nutrient medium (the bacteria cells were slightly shorter in the upper part of the sloping surface of the nutrient medium than in its lower part). This provided the opportunity for direct comparison of the test results obtained with the various substances. As a permanent comparison substance I used cane sugar which was contained as a link in each group to be simultaneously tested. - The temperature in the tests fluctuated slightly between 17° and 18° C. - For the microscope work, I used a strong microscope lamp and as a light condenser a measuring flask filled with water.

The Test Results. - Below are reproduced, in abbreviated form, only the best and most complete test series which give a clear idea of the observed effect the compounds examined had on the course of the plasmolysis and deplasmolysis in the bacteria cells. Of the experiment itself the test records show only the solution concentration of the plasmolyticum examined and the time necessary to reach the 50% degree of plasmolysis. As far as possible, the test series for one particular substance have been arranged so as to show those tests last which were made with the stop watch face down.

Tests with B. paracoli.

9 Feb. 34 Temp. 17.0° C. Age of culture: 14 hrs.[1] C = 0.19 GM.

0.2c	0.2m	0.3d	0.3m	0.4m	0.5m	
1' 30"	2' 30"	4' 45"	6' 30"	8' 15"	9' 30"	GM
1' 45"	2' 45"	5' 15"	6' 30"	8' 30"	10'	Saccharose
	45"	2' 30"	4' 45"	6' 45"	8' 45"	"
	1'	3'	5' 15"	6' 45"	8' 30"	Glucose
	45"	3'	5' 15"	8' 45"	8' 45"	"
	45"	2' 45"	5'	6' 45"	8' 45"	Mannitol
		1'	2' 15"	3' 15"	4' 30"	"
		1'	2' 30"	3' 30"	5'	Methylglycoside
						"

27 March 34 Temp. 18.0° C. Age of culture: 15 hrs. C = 0.20 GM.

(parallel tests with corresponding B. coli tests)

0.2m	0.3m	0.4m	0.5d	0.5m	
45"	2' 15"		4' 45"	8' 30"	GM
			45"	4' 15"	Saccharose
			1'	4' 30"	Methylglycoside
		45"	3'	7'	"
		1'	8' 30"	7' 15"	Rhamnose
			45"	4' 45"	"
			1'	5'	Glycohol
					"

9 Jan. 34 Temp. 17.0° C. Age of culture: 16 hrs. C = 0.17 GM.

0.1d	0.2m	0.2m	0.3m	0.4m	0.5m	0.5d	0.6m	0.6d	0.7m	
1'	3'	5' 30"								GM
	2' 45"	5'								Saccharose
			30"	3' 15"	5'	6' 45"	9'	10'	12' 30"	Erythrite
			45"	3'	5' 45"	6' 30"	9' 15"	10' 15"	12' 45"	"

10 Jan. 34 Temp. 17.0° C. Age of culture: 15 hrs. C = 0.17 GM.

0.1d	0.2m	0.2m	0.3m	0.4m	0.5m	0.5d	0.6m	0.6d	0.7m	
45"	3' 15"	5' 30"								GM
1'	3'	5' 15"								Saccharose
			30"	3' 15"	5' 45"	7'	8' 30"	9' 45"	11' 45"	Erythrite
			30"	3' 45"	5' 15"	6' 45"	8' 45"	10'	12' 15"	"

7 Feb 34. Temp. 17.0° C. Age of culture: 14 hrs. C = 0.18 GM.

0.1d	0.2m	0.3m	0.4d	0.4m	0.5m	0.5d	0.6m	0.6d	
45"	2' 30"	3' 45"	5' 45"		7' 30"	10' 45"			GM
			30"	1' 30"		3' 15"	5' 30"	7' 45"	Saccharose
			30"	1' 15"		2' 45"	5'	7' 30"	Erythrite
									"

[1] Age of culture at beginning of test.

11 Jan. 34 Temp. 17.0° C. Age of culture: 16 hrs. C = 0.18 GM.

0.10	0.20	0.30	0.40	0.50	0.60	0.70	
45″	2′15″	3′45″	6′				GM Saccharose
45″	2′45″	4′	5′30″				»
				1′15″	2′45″	3′45″	Malonamide
				45″	3′	4′	»

12 Jan. 34 Temp. 17.0° C. Age of culture: 14 hrs. C = 0.18 GM.

0.10	0.20	0.30	0.40	0.50	0.60	0.70	
45″	3′	4′	6′				GM Saccharose
30″	2′15″	3′45″	5′45″				»
				1′30″	2′45″	4′15″	Malonamide
				1′15″	2′45″	3′45″	»

5 Feb. 34 Temp. 17.0° C. Age of culture: 14 hrs. C = 0.19 GM.

0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00	
	1′30″	2′30″	5′	6′30″	9′45″					GM Saccharose
					25″	1′	1′30″	2′15″	2′45″	Malonamide
					30″	45″	1′15″	2′	2′30″	»
					15″	45″	1′30″	2′15″	3′	»

26 Jan. 34 Temp. 17.5° C. Age of culture: 14 hrs. C = 0.18 GM.

0.10	0.20	0.30	0.40	0.50	0.60	0.70	
		45″	2′45″	4′15″	6′	7′45″	GM Saccharose
		30″	2′30″	4′	5′45″	8′	»
45″	1′45″						S[1] + 0.5 GM Glycerine
1′	2′						»
45″	1′45″						»
45″	1′30″						»
30″	1′30″						S + 0.5 GM Urea
45″	1′45″						»
45″	1′15″						»
30″	1′30″						»

3 Feb. 34 Temp. 17.0° C. Age of culture: 14 hrs. C = 0.16 GM.

0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	
					30″	3′	4′	6′	GM Saccharose
					45″	2′30″	3′45″	6′	»
15″	30″	45″	1′45″						S + 0.5 GM Glycerine
17″	45″	1′15″	2′						»
15″	30″	45″	1′	2′					»
15″	30″	45″	1′30″						S + 0.5 GM Urea
25″	30″	1′	1′45″						»
18″	30″	45″	45″	1′30″					»

[1] Here and hereinafter, S means "GM saccharose."

0.10	0.12	0.14	0.16	0.18		
				15'-30"	GM	
				15"	8 + 0.8 GM	Urotropin
				30"	"	"

Tests with B. coli.

19 March 34 Temp. 18.0° C. Age of culture: 15 hrs. C = 0.19 GM.

0.00	0.02	0.04	0.06	0.08	0.10	
1'45"	2'45"	5'15"	6'45"	8'15"	9'30"	GM
1'30"	2'30"	5'	6'15"	8'	9'30"	Saccharose
	30"	2'30"	5'15"	6'45"	8'30"	"
	45"	3'15"	5'30"	7'	8'45"	Glucose
	45"	2'45"	5'15"	6'45"	8'45"	"
	1'	3'	5'15"	7'	8'45"	Mannitol
						"

27 March 34 Temp. 18.0° C. Age of culture: 15 hrs. C = 0.20 GM.

0.00	0.02	0.04	0.06	0.08	
45"	2'30"		5'	8'30"	GM
			30"	4'	Saccharose
			45"	4'	Methylglycoside
		1'	3'	7'	"
		45"	2'45"	6'45"	Rhamnose
			45"	4'30"	"
			45"	4'45"	Glycochol
					"

10 March 34 Temp. 17.0° C. Age of culture: 14 hrs. C = 0.13 GM.

0.10	0.12	0.13	0.14	0.00	0.02	0.04	
			45"	2'30"	4'15"	5'15"	GM
			45"	2'15"	4'	5'45"	Saccharose
15"	1'	1'45"					"
20"	1'15"	2'					8 + 0.8 GM Glycerine
15"	45"	1'30"					"
17"	1'	1'30"					8 + 0.8 GM Urea

Tests with B. aerogenes.

11 April 34 Temp. 18.0° C. Age of culture: 14 hrs. C = 0.22 GM.

0.00	0.04	0.06	0.08	0.00	0.02	0.04	0.06	0.08	
45"	3'30"	5'45"	8'	10'15"					GM
45"	3'15"	5'15"	7'30"	10'					Saccharose
	30"	3'45"	5'15"	6'45"					"
	45"	3'15"	4'45"	6'30"					Mannitol
	30"	3'15"	4'45"	6'					"
	30"	3'15"	4'30"	6'15"					Glucose
				45"	2'15"	5'	6'30"	8'30"	"
				1'	2'15"	5'30"	6'45"	9'	Erythrite
									"

The course of plasmolysis in bacteria is so rapid that it was not possible for me to determine for certain, in even one of the solutions, an increase in plasmolysis during the actual observation time. (In the most slowly permeating compounds -- saccharose, glucose, mannitol -- it sometimes seemed, however, as if plasmolysis had slightly increased in the first moments of observation.) This is not due, however, to a particularly high permeability of the bacteria protoplasm, but simply to the large relative surface peculiar to bacteria cells, which has the result that at the same time as the first plasmolysis is distinctly observable, or even earlier, the water to be removed osmotically from the cells has already had time to diffuse out of them and the compound to be examined to proceed through the cell membrane to the surface of the protoplast in a concentration effecting maximum plasmolysis (s. BARLUND 1929, p. 33, as well as HUBER & HOFLER 1930). For this same reason, even a stronger plasmolysis in the bacteria cells becomes reversed in a very short time, and that is precisely why the permeability of bacteria can be examined plasmolytically for slowly permeating substances only.

Simultaneously with the above experiments, I used the partial pressure method to compare the plasmolyzing effect of methyl urea with that of urea on B. paracoli. I thereby established that a solution containing 0.50 GM urea + 0.18 GM cane sugar effected a generally distinct plasmolysis whereas in a corresponding methyl urea - cane sugar solution only a 50% degree of plasmolysis was achieved. I also established that in a solution containing 0.50 GM urea + 0.18 GM cane sugar distinct plasmolysis was brought about in the cells whereas a corresponding methyl urea - cane sugar solution was not able to effect a distinctly observable plasmolysis. (The cane sugar value of the bacteria cell contents amounted to 0.18 GM.) Thus, methyl urea permeates the cells of B. paracoli noticeably faster than urea.

Some tests were also made with raffinose. They produced unexpected results which are to be discussed here in greater detail. I studied the plasmolyzing effect of raffinose on B. paracoli and B. coli. Although a 0.25-mol. cane sugar solution resulted in a clear, general plasmolysis, these studies showed that the cells in a 0.25-mol. raffinose solution (weight-normal raffinose solution to 0.50 GM as stock solution, with water added in a volume ratio 1:1) were not plasmolyzed at all but looked narrower than in water or in a corresponding cane sugar solution. In a 0.333-mol. raffinose solution as well (above stock solution with water added in a volume ratio of 2:1), no clearly observable plasmolysis could be proved, but the cells therein were definitely narrower than in water, some even were bent. A 0.50-mol. raffinose solution (weight-normal) sometimes produced violent plasmolysis the determination of which, however, was quite difficult due to the almost irritating narrowness and often bent shape of the cells. The examinations extended over 10-20 minutes, sometimes even half an hour. - The ascertained narrowing and partial bending of the cells might, however, have been caused by the low permeability of the cell membrane for raffinose (comp. FISCHER 1903, p. 30, also p. 33 of this study).

Sources of Error. - It is, of course, generally known that plasmolytic test results are always afflicted with certain errors which force us to treat them with appropriate caution. However, since these sources of error have already been thoroughly discussed in BARLUND (1929, p. 49-) I only touch on those areas which are particularly to be taken into consideration in the evaluation of results obtained with bacteria.

It is clear that the minuteness of the bacteria cells complicates the determination of their permeability in many ways. It is, for example, not possible to determine, with any great accuracy, the cane sugar value of the cell contents if only for the reason that some cane sugar has penetrated the cells even before the first examination can be made. I mentioned earlier that I evaluated the degree of plasmolysis only ocularly. It is obvious that this could not be done with the same degree of accuracy as in tests where exact counting of the plasmolyzed and unplasmolyzed cells is possible. Taking into consideration the above reservations, one glance at the test record will tell us, however, that this source of error could not have substantially influenced the results. - It might be said against the partial pressure method that the cane sugar penetrates into the cells during the test and increases the osmotic value therein. Wherever possible, I have taken this factor into account in the calculation of the results. Nevertheless, a second source of error might be linked with the partial pressure method, originating in the application of the method itself. The fact that the bacteria are directly subjected to the action of the compound solution might have the result that the plasmolyzing effect of the substance in question, particularly during the first moments of examination, is found to be lower than is usually the case because at this moment the osmotic effect of the cane sugar might not as yet have reached its climax (comp. BARLUND 1929, p. 28). During the experiments I noticed that the bacteria did not tolerate an excessively strong plasmolysis, and I therefore had to refrain from applying very high solution concentrations.

Although the determination of the permeation capacity of substances permeating rapidly or moderately rapidly is made very difficult by the minuteness of the bacteria cells, this very minuteness is nevertheless extremely useful in the study of slowly permeating substances, such as cane sugar. Particular emphasis is put upon the fact that the possibility of any detrimental influence of such sources of error as phenomena of anatonosis and catatonosis is probably very negligible even with slowly permeating substances.

Influence of Plasmolysis on Permeability. The FICK Diffusion Concept. - The original purpose of the experiments with B. paracoli, to be described here, was -- upon incentive by the work of WEBER (1932), also HUBER & SCHMIDTS (1933) -- a comparison study of "normal permeability versus permeability with plasmolysis". However, upon a later, thorough investigation of the results it became apparent that, within the limits of error, these results not only proved an equally rapid permeation (of

the cane and grape sugar as well as of mannitol) into the plasmolyzed and unplasmolyzed cells (comp. HUBER & SCHMIDT, col. 1; SCHMIDT 1936), but at the same time justified the conclusion that the penetration of these substances into the bacteria cells is merely based upon a simple diffusion process.

The method used in these experiments is demonstrated by the following example:

Experiment 1a. - The bacteria were first immersed in a 0.25-mol. (hypertonic) cane sugar solution until the stage of limit plasmolysis was achieved, which happened after 6 minutes. At an original cane sugar value of 0.19 GM in the cells, the quantity which penetrated the cells during this time amounted to 0.25 - 0.19 = 0.06 GM, corresponding to 24% of the original ratio of concentration. On this basis, and provided that the penetration of the cane sugar into the bacteria cells takes place according to the laws of diffusion, we may calculate the amount which permeated the plasmolyzed bacteria cells in 1 minute at 4.5%, in 5 minutes at 21%, and in 10 minutes at 38% of the concentration difference of the cane sugar (according to curve I in COLLANDER & BARLUND 1933, p. 27). - Experiment 1b represents a parallel experiment of the above-described experiment, with the difference, however, that the cells were first for 10 minutes put into 0.2 cubic centimeter of a 0.15-mol., i.e. hypotonic, cane sugar solution. If the permeability of these unplasmolyzed cells were the same as that of the plasmolyzed cells of the parallel experiment, 38% of 0.15 GM, or 0.057 GM, of saccharose would have had to penetrate into the cells within the time stated. To the solution were now added 0.2 ccm of a 0.35-mol. saccharose solution, thereby increasing the concentration of the compound to 0.25 GM. This increase in concentration caused plasmolysis, but already 1 minute later limit plasmolysis was reached. According to what is stated above, 4.5% of the difference 0.25 - 0.057 GM, or 0.009 GM, would have had to permeate within this time and, consequently, 0.057 + 0.009 = 0.066 GM during the total time of the experiment. The result of the experiment was 0.25 - 0.19 = 0.06 GM. This correspondence between the theoretically calculated and the experimentally determined value shows -- provided it is not a mere coincidence -- first, that the penetration of the saccharose into the bacteria cells occurs according to the diffusion concept and, second, that the permeability of the plasmolyzed bacteria cells corresponds, within the limit of error, to that of the unplasmolyzed cells.

With the help of the above example, Table 1 should be intelligible.

Experiments 1-2 were made with cane sugar, experiments 3-5 with grape sugar. In this type of experiment, I limited the reproduction to one single experiment series due to the high degree of correspondence between the various experiment series.

With regard to the grape sugar, it is to be mentioned that in a .20-mol. grape sugar solution limit plasmolysis was reached in 9 minutes,

in other words, 0.30 - 0.19 = 0.11 GM, or 37% of the initial grape sugar concentration, had penetrated into the bacteria cells within this time. Calculated from the above, the amount permeated in 1 minute is 5%, in 4 minutes 18%, in 5 minutes 22%, in 6 minutes 26%, in 10 minutes 40%, and in 20 minutes 63.6% of the concentration difference of th grape sugar.

Table 1. Comparison of Permeability in Plasmolysis and Normal Permeability of B. paracoli Cells

Test	Times	Concentration gradient	Quantity entered into cells: Calculated		Quantity entered into cells: Experimentally established
1	10'	0.19 GM	$\frac{38 \cdot 0.19}{100} = 0.007$	0.008 GM	0.30–0.19 = 0.09 GM
	1'	0.30–0.007 = 0.193 GM	$\frac{4.8 \cdot 0.193}{100} = 0.009$		
2	10'	0.19 GM	0.007		
	5'	0.30–0.007 = 0.193 GM	$\frac{21 \cdot 0.193}{100} = 0.041$	0.161 GM	0.30–0.19 = 0.19 GM
	5'	0.37–0.006 = 0.366 GM	$\frac{21 \cdot 0.366}{100} = 0.069$		
3	5'	0.30 GM	$\frac{22 \cdot 0.30}{100} = 0.066$	0.111 GM	0.30–0.19 = 0.11 GM
	6'	0.30–0.066 = 0.234 GM	$\frac{26 \cdot 0.252}{100} = 0.065$		
4	10'	0.30 GM	$\frac{40 \cdot 0.30}{100} = 0.060$	0.186 GM	0.30–0.19 = 0.11 GM
	4'	0.30–0.060 = 0.240 GM	$\frac{18 \cdot 0.240}{100} = 0.043$		
5	20'	0.30 GM	$\frac{63.6 \cdot 0.30}{100} = 0.191$		
	10'	0.30–0.191 = 0.179 GM	$\frac{40 \cdot 0.179}{100} = 0.072$	0.266 GM	0.60–0.19 = 0.41 GM
	1'	0.60–0.190 = 0.394 GM	$\frac{5 \cdot 0.394}{100} = 0.019$		

A comparison of the values shown in Table 1, calculated according to the FICK diffusion concept, and of the results obtained on an exclusively experimental basis, shows, on the whole, a surprisingly high degree of correspondence. There are some minor differences, however, which might

stem from the sources of error mentioned at the beginning, and which might have influenced the test results. If the cane sugar value of the cell fluid were, for instance, 0.20 GM or 0.18 GM instead of the 0.19 GM value used in the experiments, the permeation in the former case would progress at a slower rate, in the latter case at a faster rate, and the error in the numerical value would amount to 3.5-7%. Furthermore, should in the first experiment the 50% degree of plasmolysis set in one minute earlier or later than was assumed in the calculations (although such a large difference is hardly probable), the calculated values would be either 4-8.5% too high or too low. - The differences established fall already within these limits of error.

Corresponding results were obtained in experiments with mannitol which are not mentioned here.

Based on the above results, it is obvious that the permeation of saccharose and glucose as well as of mannitol follows the FICK concepts of diffusion. This is interesting insofar as this has been proved up to now almost exclusively (however, s. MARKLUND 1936, Fig. 15, p. 70) for glycerine and urea only as well as for substances having even greater permeation capacity (BARLUND 1929; COLLANDER & BARLUND 1933; MARKLUND 1936).

In this connection I take the liberty of touching upon a statement made by RUHLAND & HOFFMANN (1925) regarding the permeation of dissolved substances into Beggiatoa cells. The named authors write: "It was, however, established that the amount of salt absorbed into the cells per unit of time decreased as the concentration gradient increased. Therefore, the permeation process cannot merely consist in a simple diosmosis" (col. 1, p. 32; s. also p. 39 where a similar statement is made regarding aneleotrolytes). However, this in principle very important statement is based exclusively on such test results which have been considerably influenced by some sources of error not taken into consideration by some of the authors. Indeed, it must be remembered that with Beggiatoa as well as with, for example, Rhoeo, the limited water permeability of the plasm in conjunction with the diffusion inhibiting effect of the cell membrane, furthermore (with aneleotrolytes) the difference between the actual and the theoretically calculated osmotic value of the substance examined, make it seem as if the substance in question were permeating faster than is actually the case. (In the case of electrolytes, however, the effects of the last-named source of error are even more complicated due to the dissociation of the electrolytes.) Accordingly, there seems to be no reason for the assumption that permeation in Beggiatoa is not also caused by a diffusion process.

Since the final conclusions of HOFFMANN (1936), which were based on Chaetomorpha, are in close concurrence with the above, a discussion thereof might be especially appropriate here. Based on the results of the glycerine experiment, in which the permeation constancies were 0.362

after 5 minutes, 0.0942 after 20 minutes, 0.0672 after 30 minutes, and 0.0468 after 45 minutes, as well as on the basis of the results summarized in Table 7 (col. 1, p. 154), HOFFMANN states (p. 155): "With all due caution we come to the conclusion that with Chaetomorpha the plasma permeability is considerably higher at the beginning of the experiments than in their further course. The decrease seems to occur quite shortly after the beginning of the experiments."

These test results have been duplicated by BARLUND (1929, Table IX, p. 66) for example in his experiments with Rhoeo, and in the present study in experiments with Licomophora (ill. 2, p. 45) and Spirogyra (ill. 4, p. 66). Such observations, however, do not absolutely justify a final conclusion in the way HOFFMANN has done in regard to plasma permeability, because all possible sources of error must first be thoroughly clarified before any final conclusions may be reached. It has to be taken into account that still another source of error is affecting the test results even after the state of osmotic equilibrium between the cell content and the outside solution has already been reached and the concentration of the substance to be examined is practically the same as the remaining outside solution. This source of error has its origin in the difference between the determined osmotic effect of the substance examined and the theoretical value calculated according to the VAN'T HOFF concept. Thus, the permeation of different compounds into the cells used for the experiment can be determined most accurately only after the effect of the above sources of error have been eliminated for the most part. It is also highly probable that the results of HOFFMANN's endurance tests give a much more accurate picture of his test object, Chaetomorpha, than the results of his instantaneous tests if but for the reason that the latter may be most highly influenced by those sources of error which give the appearance as if each substance were penetrating the cells more rapidly than is actually the case.

In the light of this, there is no reason for assuming a divergent permeation of the other examined substances into the bacteria cells.

The Permeability Properties of the Bacteria Examined. - Table 2 shows a summary of the maximum osmotic coefficients of the compounds examined for Beggiatoa mirabilis and Bacterium paracoli. Based on the limit concentration values shown by SCHONFELDER (1931, Tab. 3, p. 437-442) I calculated the maximum osmotic coefficients for Beggiatoa (saccharose = 1.0).

A comparison of the values for Beggiatoa and B. paracoli reveals great differences as to the permeability conditions. Thus, the behavior of urea and methyl urea is quite opposite in the two objects. Furthermore, the permeation of urea in comparison with glycerine is distinctly faster in Beggiatoa than in B. paracoli. In Beggiatoa, antipyrin permeates more slowly than glycerine but more rapidly than glycerine in B. paracoli (comp. FISCHER 1930, p. 25).

Table 2. Maximum Osmotic Coefficients for Beggiatoa Mirabilis (Calculated according to data by SCHONFELDER) and for Bacterium Paracoli

Beggiatoa mirabilis		Bacterium paracoli	
Urea	< 0.001	Methyl urea	< 0.020
Methyl urea	0.020	Urotropin	0.020
Glycerin	0.022	Urea	0.180
Malonamide	0.029	Glycerine	0.180
Antipyrine	0.080	Malonamide	0.633
Glycohol	0.100	Erythrite	0.760
Erythrite	0.200	Methylglucoside	0.806
Glucose	0.364	Glycochol	0.845
Mannitol	0.364	Rhamnose	0.890
Rhamnose	0.400	Glucose	0.905
Saccharose	1.000	Mannitol	0.905
		Saccharose	1.000

The differences in permeability observed are caused above all by the known fact that permeation of compounds into the cells of Beggiatoa is first of all determined by particle size, lipin solubility being less significant here, whereas with B. paracoli it seems on the contrary that permeation depends chiefly upon lipin solubility (methyl urea and urotropin permeate more rapidly than urea, methylgluside more rapidly than glucose). The experiments do not give any exact information on the pore permeability of the plasm membrane of B. paracoli, but it does not appear to be greater than in most other plant protoplasts.

Although the above comparison has already given us a general idea of the permeability conditions in B. paracoli, a closer comparison of the permeability properties of this bacterium and those of Chara ceratophylla seems nevertheless appropriate since in this way the obtained results may be directly incorporated into the comparative permeability research. I have chosen Chara cells as the main comparison object in my present study because the test results obtained with them are relatively certain, because they represent the most widespread permeability type, and because the aggregate of substances examined - with few exceptions - formed part of the compounds used in the experiments on Chara.

In proceeding to a quantitative comparison of the permeability series of the last-mentioned objects, it is first of all necessary to clarify the reasons on which such a comparison is based. As early as 1902, OVERTON explained how important it is also to include the aspect of cell size when evaluating plasma permeability. However, not until COLLANDER & BARLUND (1933, p. 53) had emphasized to what erroneous results permeability comparisons may lead when carried out without taking into account the relative surface development of the protoplasts, did an ever increasing

number of investigators (HOFLER 1934 a, b; in a way also HOFMEISTER 1935; WAHRY 1936; MARKLUND 1936) base their comparison on the permeability as calculated per ratio of surface unit of the protoplasm. As the objects used in my investigation comprised a wide variety of cell sizes, this aspect was particularly important for me.

Table 3 shows, from among the compounds I examined, the calculated permeation constancies for all those substances for which it was possible to determine, with reasonable certainty, a temporary plasmolytic coefficient at a given time (t), such coefficient being named w-value in accordance with COLLANDER & BARLUND (1926). Here, as everywhere else, I strived to choose the w-value in such a way as to permit as correct as possible a picture of the penetration of the examined compounds into the cell. - In calculating the permeation constancies I used in all instances the hour as time unit and the square centimeter as surface measure. The bacteria cells examined are cylindric, their diameter is 0.0001 cm. Disregarding the cell ends in the calculation of permeation, a diffusion surface of 40,000 square centimeter corresponds to a cubic centimeter of cell volume, and P therefore equals P' = 40,000.

A glance on the last columns of the table shows an unexpectedly great similarity in the permeability properties of the bacterium and of Chara. This observation is all the more satisfying as we are conscious of the otherwise large differences in the two objects. It is also to be mentioned that the permeability properties of the two objects have been determined according to quite different methods: with Chara by direct microchemical analyses of the penetrated quantities of substance, with B. paracoli by means of the plasmolysis method which is regarded as doubtful or has even been completely rejected by several investigators (s. COLLANDER & BARLUND 1933, p. 4).

Table 3. Permeability Series for B. paracoli compared with that for Chara (according to COLLANDER & BARLUND)

Bacterium				Chara	Substance	For glycerine = 1	
w	t (Min.)	P'	P·10⁴	P·10⁴		Bacterium	Chara
0.00	1.0	110	28	40	Urea	1.0	5.4
0.00	2	84	21	7.4	Glycerin . . .	1	1
0.0	1	42	11	1.4	Malonamide . .	0.00	0.19
0.0	6	6.0	1.7	0.00	Erythrite. . .	0.001	0.000
0.07	4.0	5.4	1.4	<0.0	Methylglucoside	0.007	<0.00
0.07	5	4.0	1.0		Glycocohol. . .	0.007	
0.07	7	3.4	0.00		Rhamnose . . .	0.004	
0.00	8.4	3.0	0.00	<0.0	Glucose . . .	0.000	<0.00
0.00	8.70	3.1	0.70	<0.0	Mannitol . . .	0.007	<0.00
0.00	9.70	3.0	0.70	<0.0	Saccharose . .	0.000	<0.00

Closer investigation shows quantitative differences in the permeability series of the two test objects. It is difficult to determine, however, whether these differences are actually present or merely stem from test errors. It must be mentioned in this connection that Chara cells are relatively ill suited for observing the permeation capacity of very slowly permeating substances, while all the test results obtained with bacteria, particularly those obtained with more rapidly permeating substances, are rather inaccurate due to the nature of this test object.

In this connection let us also point to the test results for glycocohol which are in close agreement with results other investigators have obtained for this substance as well as for other amino acids (SCHONFELDER 1931; ULRICH 1934).

3. ORIENTATION TESTS WITH GRAM-POSITIVE BACTERIA

After completion of the above experiments, and in order to gain personal experience with those bacteria generally considered unplasmolyzable (s. FISCHER 1903, p. 25), I also conducted some orientation tests with the following gram-positive bacteria: Bacterium megatherium, B. mesenterious fuscus, B. cereus, Bacillus subtilis, and Streptothrix hominis "Goteborg". The result was that B. megatherium and Streptothrix hominis did not become plasmolyzed in cane sugar solutions of 0.5 GM and 1.0 GM nor in a 1.0-mol. KNO_3 solution, but looked narrower in the two latter solutions than in water and, at the same time, almost distressed. Neither did B. mesenterious become plasmolyzed in the above-mentioned solutions, but the behavior of the bacteria in the various solutions showed a difference insofar as the cells moved lively in the weaker saccharose solutions whereas in the stronger solutions part of the cells -- in the KNO_3 solution most of the cells -- ceased their movements. With B. cereus, there appeared to be an indistinct, plasmolysis-like contraction; this appearance was more distinct in the KNO_3 solution. In the stronger cane sugar solution, B. subtilis showed a nice, clear plasmolysis (comp. FISCHER 1895, p. 32, and 1903, p. 25; MIEHE 1931, p. 672). However, I conducted more detailed plasmolysis experiments only with B. subtilis. The results of these experiments are outlined below:

The method of culture as well as the other test conditions were the same as before. In 1.0-mol. cane sugar and grape sugar solutions clear plasmolysis set in although it was slightly less pronounced in the latter two solutions. Plasmolysis in the cells was maintained in full force during the entire test time (45 minutes). In a saccharose solution of 0.75 GM, plasmolysis was weaker and the degree of plasmolysis 50% or slightly above. In correspondingly strong grape sugar and mannitol solutions, plasmoly is was weak and the degree of plasmolysis clearly below 50%. Even after 35 minutes, plasmolysis had not yet set in. Neither was the 50% degree of plasmolysis reached in a 1.0-mol. glycocohol solution. 1.0-mol. and 1.5-mol. erythrite solutions effected no plasmolysis whatever.

Since the above tests showed that plasmolysis in the cells of B. subtilis did not become reversed nearly as rapidly in the cells of B. subtilis as in the previously examined bacteria, I undertook two lengthy experiments with a saccharose solution in order to clarify this behavior:

Test Series I. - 19 April 1934. Temperature: 18.5° C. Age of Culture: 14 hours.

The cells were at first allowed to remain in a 0.25-mol. cane sugar solution for 10, 20, 30, and 40 minutes, respectively, and were then transferred into a stronger cane sugar solution of 1.0 GM. The first observation was made after 1 minute. The result was that the cells in all tests became plasmolyzed in exactly the same manner as if they had been put directly into the stronger cane sugar solution. In a simultaneously conducted parallel test, the cells remained for equally long periods of time in a 0.5-mol. (hypotonic) cane sugar solution the concentration of which was subsequently increased to 1.0 GM in one operation. General plasmolysis was observed after 1 minute. The test results seem to indicate that in 40 minutes the cane sugar does not penetrate, at least not in detectable amounts, into the cells of B. subtilis.

Test Series II. - 20 April 1934. Temperature: 18.0° C. Age of Culture: 14 hours.

In a 0.75-mol. cane sugar solution, the degree of plasmolysis slightly exceeded 50%. The cells first remained for 3 minutes in the above-mentioned solution, the concentration was then gradually decreased to 0.375 GM cane sugar, and the cells were allowed to remain therein for 10 minutes. The concentration was then increased to 0.75 GM, after which a degree of plasmolysis of slightly above 50% set in again. In a second test, the cells first remained for 30 minutes in a 0.75-mol. cane sugar solution and remained plasmolyzed during this entire time at slightly above 50%. A decrease in the concentration to 0.5 and 0.375 GM cane sugar, respectively, resulted in complete deplasmolysis in both cases. After 10 minutes, the concentration was again increased to the initial value. Result: 50% plasmolysis. Consequently, the change from plasmolysis to deplasmolysis does not appear to have any influence on the permeation of cane sugar.

In summary, we may say with regard to the tests made with gram-positive bacteria that, of the bacteria examined, B. cereus and B. subtilis became plasmolyzed in 1.0-mol. cane sugar and KNO_3 solutions. It seems, consequently, that the established non-plasmolyzability of gram-positive bacteria is caused by a tighter than usual cohesion of protoplast and cell membrane rather than by any great permeability of such cell membrane (comp. FISCHER 1903, p. 25-). The test results on B. subtilis show furthermore that saccharose, glucose, and mannitol do not seem to permeate in detectable quantities into the cells during the test period of 40 minutes; in

other words, it would appear that the cells of B. subtilis are much less permeable to saccharose, glucose, and mannitol than the examined gram-negative bacteria. Obviously, the interpretation of these peculiar test results still rests, for the present, on a quite uncertain basis.