Studies in Permeability.

IV. The Action of Various Organic Substances on the Permeability of the Plant Cell, and its Bearing on Czapek's Theory of the Plasma Membrane.

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With fifteen Figures in the Text.

INTRODUCTION.

WHEN plant tissue is immersed in aqueous solutions of various substances part of the contents of the cells diffuses out into the external liquid as a result of changes produced in the permeability of the cell. Czapek (1, 2, 3) in an investigation of the question comes to the conclusion that an important relation exists between the surface tension of aqueous solutions in contact with air and their power of producing exosmosis of the cell contents, and from his conclusions in this respect builds up a theory of the plasma membrane. He alleges that a solution will not produce exosmosis from a plant cell unless its surface tension against air is reduced to a certain definite value. Czapek determined the limiting concentration which is just capable of producing exosmosis from plant cells of certain cell substances whose presence can be easily demonstrated. The solution in this limiting concentration should have the same critical surface tension independent of the substance, and in most of Czapek's experiments he concluded that this was the case. He says, 'Surface active substances do not alter the diosmotic properties of the plasma membrane before they act injuriously on the cell on account of their surface tension' (Czapek 3, p. 1).

Several authors have already subjected Czapek's work to considerable criticism, and indeed it seems strange to attempt to explain the properties of a highly complex organization like the plasma membrane in terms of one physical property.

A very brief consideration is required to make it clear that in the system produced by immersing plant tissue in an aqueous solution of a substance several factors must necessarily come into play. For instance, if we make the assumption that the change in permeability of the cell is due

to chemical combination between the solute in the external medium and some substance of the plasma membrane, the rate at which the permeability changes will depend upon the active masses of the reacting substances. We know also that according to the principle of Willard Gibbs the lower the surface tension at the interface, the greater will be the concentration of solutes there. The actual rate of the reaction will nevertheless be controlled by the laws of mass action, although the lowering of the surface tension may increase the active mass of the dissolved substance at the interface and so increase the rate of reaction and, in consequence, the rate of change of permeability. On the other hand, if the surface tension of the solution is raised, chemical action will still take place, though it may be at a reduced rate. But other physical factors may be of importance in determining the rate of reaction besides surface tension. Thus, if the reacting substance is removed by chemical action faster than it can be replaced by diffusion, this latter factor will assume greater prominence in determining the rate of reaction.

However, on account of its undoubted complexity the plasma membrane cannot be assumed to be merely a system in which permeability alters simply as a result of chemical action. So, for instance, Schryver (8) thinks of the substances as cemented together in a gel, on the alteration of which the phenomena of permeability changes mainly depend, and in a later paper (9) he points out how many physical factors influence the state of aggregation of the gel.

Czapek investigated the exosmosis of tannin substances from the 'sub-epidermal mesophyll' cells of *Echeveria* leaves and from other objects. When treated with various reagents, notably with an aqueous solution of caffeine, the tannin in cells is precipitated in a characteristic and easily recognizable manner. Sections of *Echeveria* leaves, for example, are placed in 50 c.c. of the solution under investigation, and removed when equilibrium has been attained and tested for the presence of tannin. If tannin is absent it is assumed that exosmosis has taken place. There is no definite time in which equilibrium is attained; this has to be found in each case. 'Bei fortgesetzter Beobachtung lässt sich die Zeit leicht ermitteln, in welcher ein länger dauernder Gleichgewichtszustand hinsichtlich der Exosmosegrenze besteht, und diese Grenze stimmt mit der theoretischen Tensionsgrenze in der Regel befriedigend überein.'

Plant organs containing coloured cell-sap were also tried, for the exosmosis of anthocyan can be observed without the aid of a microscope. But most of these objects had the disadvantage that they were damaged after less than 24 hours' immersion in solutions of higher surface tension than the critical value. So 'resistant objects' such as *Echeveria* leaves, in which 'secondary damage' does not occur, were chiefly used by Czapek throughout the investigation. In some cases these were left for 24 hours, and even for 48 hours, for the equilibrium condition to be reached.

The surface tension of the various solutions used was measured by means of a capillary manometer, the essential feature of which is the measurement of the pressure just required to force a bubble of air through a capillary tube containing the liquid under examination. From this may be calculated the surface tension of the solution in contact with air. Of course what we are actually concerned with in exosmosis is the surface tension between the solution and the protoplast, which may be a very different thing. But because 'all the experience we possess' indicates that increase in concentration of the solution external to the protoplast causes a lowering of surface tension, and since increase of concentration of these same solutions also brings about a lowering of their surface tension against air, Czapek neglects the essential difference between the conditions in his experiments with the plant and the conditions under which the surface tension is measured.

A large variety of organic substances was used by Czapek in these experiments, including a series of monohydric alcohols, glycol, glycerine, esters, &c. The general conclusion is reached that exosmosis only takes place from the cell when the surface tension of the external liquid when in contact with air is reduced to 0.68 times the surface tension of pure water against air. From this rather remarkable conclusion the surprising deduction is made that the surface tension of the outer layer of the protoplast in contact with air must also have this value. Since Czapek found by means of his capillary manometer that emulsions of various fatty substances occurring in plants, unsaturated triglycerides such as castor oil, linseed oil, and triolein, have the same surface tension, and that the surface tension can never be reduced below this value of about 0.68, he further concludes that the surface-tension properties of the plasma membrane are chiefly due to the presence in the outer layer of the protoplast of such unsaturated triglycerides. These neutral fats are supposed to be present in the form of an emulsion with water, the globules being coated with a layer of soap which acts as a protective colloid. Czapek admits that he has not disproved Overton's view that lecithin and cholesterine may be the essential substances in the plasma membrane, and he is also prepared to agree that there may be some protein present as well. The important substances, however, in his opinion are the neutral fats.

Czapek's theoretical considerations have already been subjected to severe criticism, to which, as we shall draw attention later, he has been unable to make any adequate reply. But, apart from his theory, it becomes of interest to examine whether it is permissible for him to draw the conclusion he does from his experiments. The method he used was laborious and crude, and he regards the measurements he made as referring to equilibrium conditions which he supposed to have become established in the systems he investigated. It is necessary, therefore, to reinvestigate the

problem, and to repeat some of his experiments with more exact methods, which will at the same time enable one to obtain an insight into the kinetics of the changes taking place. This we have done in the experiments described in this paper, and we shall show as a result of them, that not only is the theory founded by Czapek upon his experimental results untenable, but that the facts on which he bases his theory are merely illusions of experimentation due partly to the crudity of the method employed, and partly to a selection and arbitrary arrangement of experiments.

We do not intend in this paper to enter into a general discussion of the permeability of the cell, nor to differentiate between the permeability properties of different tissues. Nevertheless we would point out that the chemical composition and physical properties of membranes in different tissues are not likely to be identical, and their permeability properties are consequently not likely to be expressed by one general law.

In order to avoid discussion which available information does not justify, we use the generally accepted nomenclature, including the term 'plasma membrane'. By this latter expression we mean that part of the cell which is concerned in the phenomena of permeability, without reference to its actual location in the cell.

METHODS USED IN THE PRESENT INVESTIGATION.

In this investigation we have employed a method for studying permeability changes which is more sensitive and of more general application than the methods of earlier workers, and which at the same time allows the changes taking place to be followed easily from time to time. This method consists essentially of measuring the rate of exosmosis of electrolytes from plant tissue by means of measurements of the electrical conductivity of the solution in which the tissue is placed. In former investigations in which we have used this method the solution external to the tissue has been an electrolyte, and so changes in the conductivity of the external solution have been the resultant of diffusion of electrolytes into the tissue from the solution and diffusion of electrolytes out of the tissue into the solution. In the present investigation the substances examined are all practically non-electrolytes, and the electrical conductivities of solutions of them are almost identical with that of the conductivity of the water used in preparing them. Hence the increase in conductivity will give a much surer measure of the extent of exosmosis than when the effect of acids or salts is under investigation. It is, however, necessary to make allowance for the lowering of conductivity due to the presence of non-electrolytes, and this has been done in the cases cited in the following pages.

A brief account of the method has been given in a previous paper (10), but we may here take the opportunity of describing the method in more detail.

A suitable quantity of the solution under investigation is placed in

a stoppered vessel and a definite amount of plant tissue is added. The vessel is kept at a constant temperature in a thermostat, and the electrical conductivity of the liquid measured at suitable intervals by Kohlrausch's method. From the numbers obtained in this way the relation between time and exosmosis of electrolytes may be exhibited graphically in a curve. In the majority of our experiments we have used potato tissue. Cylinders of potato were cut out from the tuber by means of a cork-borer, and these were cut up into discs of a definite thickness. These discs had a diameter of 17 mm., a thickness of about 1.75 mm., and a weight of about 0.45 gm. After they were prepared they were washed for an hour in running tap-water, rinsed rapidly in distilled water, and then dried with blotting-paper. In each experiment twenty discs were immersed in 50 c.c. of solution. The experiments were carried out at a constant temperature of 20° C., and the conductivity measurements were all made at the same temperature.

In order to correct the results obtained for the depression in conductivity due to the presence of non-electrolytes in the solution, the following method was adopted. Thirty-six gm. of potato discs were boiled in about 95 c.c. of water and the extract so obtained made up to 100 c.c. This should give a solution of approximately double the strength of the external solutions when equilibrium is attained. Quantities of 10 c.c. of this were then taken and made up with either pure water or a solution of the non-electrolyte to 20 c.c. The conductivity of the solutions so obtained was measured, and by this means curves were obtained showing the depression of the conductivity brought about by various concentrations of the non-electrolytes employed. With lower concentrations of cell exudate the absolute decrease in conductivity resulting from the presence of non-electrolytes is smaller, the lowering being approximately proportional to the concentration of electrolyte. These results agree with the extensive observations of Jones and his collaborators (5). By means of these curves the numbers actually obtained in the experiments were corrected before plotting the curves between the time and the exosmosis of electrolytes. In the case of methyl, ethyl, and to a less extent propyl alcohol, where high concentrations of alcohol were used, the correction that has to be applied in this way is large (see Figs. 4 and 5), but in other cases, though it is much smaller, it is as well not to neglect it.

As the discs are liable to vary in thickness and weight some experiments were made in which discs of various thickness and weight were used with the same quantity of external solution in each case. The liquid used was an aqueous molecular solution of pyridine; in each case 50 c.c. of this solution were employed with twenty discs. The weights of the twenty discs in five cases were 3.8 gm., 4.1 gm., 5.8 gm., 9.4 gm., 14.74 gm. The curves given in Fig. 1 show the relation between the time and the exosmosis of electrolytes in the five cases. It will be noticed that in each case there is a rapid exosmosis of electrolytes lasting over the first few

hours, and then the rate of exosmosis rapidly falls off until the quantity of electrolytic exudate remains constant, which means that equilibrium has become established between electrolytes in the external solution and the plant tissue. The rate of exosmosis during the first period does not vary greatly with the weight of tissue, being practically proportional to the

FIG. 1. The exosmosis of electrolytes from various quantities of potato in molecular solutions of pyridine.

surface. There is, however, a very great difference in the final value of the conductivity when different weights of tissue are used. The following table shows the relation between the final value of the conductivity and the weight of tissue used. The numbers in the third column have been obtained by assuming that when equilibrium is obtained there is the same concentration of electrolytes in the solution and the tissue.

<i>Weight of tissue.</i>	<i>Final value of conductivity.</i>	<i>Total quantity of electrolytes in the system.</i>	$\frac{E}{W}$.
(W)		(E)	
3.8	258	275	72
4.1	300	322	79
5.8	399	440	76
9.4	520	605	64
14.74	802	1027	70

Thus the total exosmosis varies with the weight of tissue used. In the experiments to be described the weight of discs varied somewhat, and in some cases the deviation from the mean was as much as 5 per cent. of the mean value. Hence variations in the values for the final conductivity of the solutions are no doubt due to variations in the weight of tissue employed. It is, however, only the final value of electrolytic exudate which is affected; slight variations in the weight of discs used will scarcely affect the quantity of exosmosis of electrolytes before the final condition is reached, and any differences in the earlier part of the process must be due to other causes.

We have used potato in our experiments, as potato tubers yield a very uniform tissue, and individual variations are likely to be less than with most other plant organs. But the method can be used for almost any plant tissue. We ourselves have used discs of beetroot and other fleshy organs similar to those of potato, and we have also employed leaves of various kinds, as well as roots of living plants, and we have found the method to answer as well in these cases as with potato.

In the cases described in this paper we have chiefly used concentrations of reagents which will produce irreversible changes in the permeability of the cell, i. e. lead to the death of the tissue. In these cases the morphological structure of the tissue employed does not introduce much variation in the results, but if very low concentrations are used, such as would not for a long time lead to irreversible permeability changes, then the morphological structure and chemical composition become of importance. This is easily observed by comparison of potato and carrot. With these reversible changes of permeability and their bearings on theories of permeability we hope to deal more in detail in a later paper.

It is interesting to compare results obtained by this method with some others that have been employed in work on permeability. Two methods that have found favour earlier we have tried in this investigation. The first of these is the exosmosis of the red pigment from anthocyan-containing cells. For this purpose the root of the red beet has been much used in the past. As the cell membranes become more permeable the red-coloured substance diffuses out, and the external solution becomes coloured with it. It is possible to follow the rate of exosmosis by measuring the colour intensity at different times by means of a colorimeter. By following both the increase in colour intensity of the external solution and its increase in conductivity

it can be readily shown that there is a rough parallelism between the two. But the measurement of the electrical conductivity can be made much more accurately than colorimetric measurements.

In the following experiment twenty discs of red beetroot, weighing altogether 10 gm., were placed in 50 c.c. of solutions of isoamyl alcohol (Kahlbaum) of various strengths. The conductivity was measured at various intervals of time and the colour intensity estimated. The colour intensity was compared with that of an extract obtained by boiling 10 gm. of beetroot with water and diluting down to 50 c.c. with slightly acid water. The following results were obtained, which are shown graphically in Fig. 2.

FIG. 2. Exosmosis of electrolytes from beetroot in solutions of isoamyl alcohol of various concentrations.

Ten gm. beetroot (twenty discs) in 50 c.c. 0.2 m. isoamyl alcohol.

Time in hrs. and minutes	Conductivity. (Sap extract = 900)	Colour. (Sap extract = 1)
0.15	120.8	0.5
0.50	412.4	1.0
1.25	649.5	1.0
1.55	766.0	1.0
3.35	795.5	1.0
19.15	782.0	1.0

0.12 m. isoamyl alcohol.

Time.	Conductivity.	Colour.
1.0	40.8	less than 0.05
3.45	128.8	0.1
19.15	636.5	1.0
48.0	750.0	1.0

0.08 m. isoamyl alcohol.

Time.	Conductivity.	Colour.
2.30	55.2	very slight
19.30	220.2	0.2
45.0	578.0	0.33

0.04 m. isoamyl alcohol.

Time.	Conductivity.	Colour.
2.30	23.5	almost colourless.
19.30	120.0	0.10
43.30	484.0	0.14
67.30	719.5	

It is clear that there is a rough parallelism between the exosmosis of electrolytes and of the sap pigment, but the measurement of the electrolytes can be accomplished much more easily and exactly than that of the pigment.¹

Moreover, certain substances may react with the pigment and so change the colour, when colorimetric measurements would be rendered difficult or impossible.

Incidentally it appears from a comparison of the results obtained with potato and beet that the latter contains somewhat more electrolytes than the potato.

Another method which may be used depends upon the change of colour of the chlorophyll in leaves following on permeability changes in the cell which bring acids in contact with the chlorophyll. Chlorophyll when acted upon by weak acids gives derivatives of a yellowish or yellowish-green colour. The leaf of the Wood Sorrel (*Oxalis acetosella*) is especially useful in this respect, as the colour change from a bright green to a bright yellow is particularly easily observed.

The method has the disadvantage that it cannot be used kinetically. The only method of comparing the effects of different conditions is to note the time taken for the colour change to take place. It is, again, of very limited application as regards the tissue employed for experimentation. Further, any substance which neutralizes the acid in the cell-sap will prevent the colour change.

¹ Nevertheless, in the lower strengths of alcohol the pigment obviously diffuses out much less rapidly than the electrolytes. The question may arise here of differential permeability of the cell to different substances. With this question we may have occasion to deal later.

Thus, a molecular solution of pyridine and an 0.2 m. solution of isoamyl alcohol both produce rapid exosmosis of electrolytes from the cell; but, owing no doubt to the basic character of pyridine, leaves of *Oxalis* in the pyridine solution do not turn yellow, even when the greater part of the electrolytes have passed out of the cell. In the solution of amyl alcohol, on the other hand, the leaves turn yellow almost immediately after immersion in the solution.

The numbers obtained in the experiment described below show this.

2.5 gm. of leaves of *Oxalis acetosella* were placed in 50 c.c. of the following solutions:

Pyridine, m.

Isobutyl alcohol, 0.5 m.

Isoamyl alcohol, 0.2 m.

The conductivity of the external solution was measured from time to time and colour change in the leaves noted. The results obtained are given in the following table, and are also shown graphically in Fig. 3.

FIG 3. Exosmosis of electrolytes from leaves of *Oxalis acetosella*. A, in 0.2 m. isoamyl alcohol; B, in 0.5 m. isobutyl alcohol; P, in m. pyridine.

Pyridine, m.

Time. hrs. mins.	Conductivity.	Colour.
1.0	133.6	
2.0	284.2	
3.05	379.8	
3.30	397.6	
4.50	400.9	
5.50	448.8	
6.35	465.3	no general yellowing

Isobutyl alcohol, 0.5 m.

Time. hrs. mins.	Conductivity.	Colour.
0.30	33.8	
1.15	72.1	leaves turning yellow
1.35	119.8	leaves strongly yellow
2.35	127.3	
3.15	309.0	
5.0	412.6	

Isoamyl alcohol, 0.2 m.

Time. hrs. mins.	Conductivity.	Colour.
0.30	108.3	leaves strongly yellow
1.0	198.4	
1.25	242.4	
2.20	298.8	
3.0	500.0	
4.10	548.0	

We have also tried leaves of *Pelargonium*, in which a change to olive-green occurs with similar results. But it will be obvious from the examples with *Oxalis*, which gives a very marked colour change, that the method of electrical conductivity measurement is a much more accurate one for following permeability changes than the one depending on colour changes in the leaves. Moreover, it is independent of the nature of the substances in the external solution, whereas whether the colour change takes place in the leaf or not may depend on the chemical constitution of the substance in solution and have no relation to permeability.

Czapek's method, which consists in testing for the presence or absence of tannin in the leaf by means of caffeine, is very similar to the method based on the colour change in leaves. It has all the disadvantages of that method, and the additional one that it involves the use of a microchemical test in place of a very clear colour change easily observable by the naked eye.

For studying permeability changes in the cell in general, the electrical conductivity method here described is to be recommended in preference to earlier methods on account of its accuracy and its general applicability to all plant tissue and all non-electrolytic external solutions, and because it enables the changes taking place to be investigated in a manner impossible with the older methods. It must, however, be clearly understood that the results obtained by its means have direct reference to the permeability of the cell only as regards electrolytes; the results do not necessarily hold, and probably do not hold, for all substances.

In experiments with distilled water and low concentrations of substances, which are continued over a considerable time, a complication is introduced by secondary reactions, such as the effect of substances produced by bacterial action in the medium. We have therefore in these cases also employed another method in which the liquid is constantly renewed, whereby such secondary reactions are eliminated. But during the first twenty-four hours the two methods give practically identical results with the material we have employed. We may therefore postpone the description of this method until a later occasion.

EXPERIMENTAL RESULTS.

In all experiments recorded in this section twenty discs of potato, weighing altogether about 9 grm., were kept in 50 c.c. of solution at 20° C., at which temperature also the conductivity measurements were made. The experiments were generally continued for 24 hours, but in some cases, of which mention will be made, the time of experimentation was considerably longer. The conductivity measurements were all corrected for depression in their value caused by the presence of non-electrolytes in the external solution in the manner described in the previous section of this paper. The conductivities are stated in arbitrary units. To obtain the actual specific conductivities the values given have to be multiplied by a constant.

When exosmosis is complete and equilibrium established between the electrolytes in the external solution and inside the plant cells, the corrected conductivity measurement is about 550, though, as already mentioned, this value is liable to variation corresponding with the weight of potato used.

As we have shown in a previous paper, electrolytes diffuse out from cells even when they are surrounded by distilled water. After 48 hours' immersion in conductivity water of twenty discs of potato, the conductivity of the liquid has increased to 144.8, only about a quarter of the value for complete exosmosis. The curve for distilled water is given in Figs. 4 and 6.

The substances employed in this investigation were the following :

Methyl alcohol	Hopkin and Williams, free from acetone.
Ethyl alcohol	'Absolute alcohol.'
Normal propyl alcohol	Kahlbaum.
Isobutyl alcohol	Kahlbaum.
Isoamyl alcohol	Kahlbaum.
Secondary I. Octyl alcohol, $\text{CH}_3(\text{CH}_2)_5\text{CHOH}\cdot\text{CH}_3$	Kahlbaum.
Acetone	British Drug Houses, Analytical Reagent.
Chloroform	Hopkin and Williams, pure.
Ether	Tyrer, S. G. 0.717.
Chloral hydrate	Kahlbaum.

Urethane	Kahlbaum.
Aniline	Hopkin and Williams, pure.
Pyridine	Hopkin and Williams, pure.

I. The Monohydric Alcohols.

The effect of methyl alcohol on exosmosis of electrolytes was examined in a large number of concentrations varying from $\frac{m}{100}$ to 10 m.

The exosmosis during the first 24 hours is exhibited in Fig. 5. It will

FIG. 4. Exosmosis of electrolytes from potato immersed in solutions of methyl alcohol of various concentrations. The conductivity values are those actually measured uncorrected for the depression in their value resulting from the presence of the methyl alcohol.

FIG. 5. Exosmosis of electrolytes from potato in solutions of methyl alcohol of various concentrations. The conductivity values in this and succeeding figures are corrected for the presence of non-electrolytes.

be observed that throughout, with increasing concentration, there is an increased rate of exosmosis. From the lowest concentrations up to molecular concentration the permeability is increased very little with increasing concentration; nevertheless, in these lower concentrations increase in

strength of the alcohol does result in increased exosmosis. In a concentration of 2 m. methyl alcohol the permeability increases at a considerably more rapid rate than in the case of a solution of m. concentration, though the difference is only noticeable after three or four hours' immersion in the solutions. A solution of 3 m. methyl alcohol produces more marked exosmosis from the first, but after three hours the rate of exosmosis gradually increases for some hours until this rate is reduced owing to reduction in the concentration of electrolytes inside the cell, the maximum exosmosis not being reached till about 16 to 20 hours.

With 4 m. methyl alcohol the same state of affairs is observable, the permeability increasing more than in the case of 3 m. from the beginning. The marked increase in the rate of exosmosis begins sooner, after about an hour and an half, and the total exosmosis approximates to the maximum

FIG. 6. Exosmosis of electrolytes from potato in solutions of ethyl alcohol of various concentrations.

after 9 or 10 hours' immersion. Higher strengths of alcohol show an increased rate of exosmosis during the early stages, the preliminary period during which the permeability increases comparatively slowly being reduced in duration with increasing concentration of alcohol. In 10 m. methyl alcohol, as the accompanying curves show, the preliminary stage must occupy considerably less time than 20 minutes.

The most numerous series of concentrations was used in the case of ethyl alcohol. The following were the solutions employed: 0.0, 0.75 m., 1.0 m., 1.5 m., 2 m., 4 m., 6 m., 8 m. The results are shown in Fig. 6. They are exactly similar to those obtained with methyl alcohol, except that a smaller molecular concentration is required to produce the same effect on the permeability of the cell. Thus a 1.5 m. solution of ethyl alcohol produces changes in permeability of the cell similar to those produced by

a 3 m. solution of methyl alcohol. The curves show how, as a result of the increased permeability produced by increased concentration, the maximum value of the exosmosis is reached sooner with increasing concentration. Although the curves are only given for the first 24 hours, measurements were made over a considerably longer period. The following table shows that eventually complete exosmosis of electrolytes takes place even with considerably weaker solutions than 2 m., which, according to Czapek, is the limiting concentration above which exosmosis takes place and below which it does not :

Concentration of solution.	Conductivity after				
	2 hrs.	4 hrs.	12 hrs.	24 hrs.	48 hrs.
8 m.	466	525			
6 m.	450	516	538		
4 m.	430	552	562		
2 m.	194	464	570	570	
1.5 m.	33	90	307	509	
m.	20	38	82	156	390
0.75 m.	16	29	59	110	370
Distilled water	10	21	50	79	141

The above table shows that it is impossible to speak of a critical concentration required to produce exosmosis of the cell contents. The rate of exosmosis, indeed, depends on the concentration, but diffusion out from the cell takes place in all concentrations. Czapek's value of 11 per cent. by volume (about 2 m.) as the critical concentration required to produce exosmosis is therefore meaningless. A different result is obtained by Czapek's method according to the time one supposes is required to produce equilibrium conditions. Even with distilled water, exosmosis continues slowly until bacterial action sets in and measurements no longer have reference to the simple action between potato tissue and water. So that by assuming equilibrium to be established after any selected time, any desired value can be obtained as a value of the critical concentration required to produce any particular amount of exosmosis.

But one matter exhibited by Czapek is confirmed in this investigation, which is that any member of the homologous series of primary alcohols has a greater effect on exosmosis than a lower member of the series in the same concentration.

Thus experiments made with normal propyl alcohol show that this alcohol in 0.6 m. concentration is equivalent in regard to its action in producing exosmosis to ethyl alcohol of 1.3 m. concentration. The general course of events is exactly similar to that with the two lower alcohols. The accompanying curves (Fig. 7) show the exosmosis during the first 24 hours for concentrations of normal propyl alcohol from 0.4 m. to m. It should be noted, however, that in the case of the 0.4 m. solution, the maximum exosmosis was approached after 48 hours, although from an inspection of the curves for the first 24 hours this differed little from that

for distilled water. Distilled water after 48 hours only shows a conductivity of about a quarter of this value.

The experiments with higher alcohols, isobutyl alcohol, isoamyl alcohol, and a secondary octyl alcohol, all gave regular results, which might be ex-

FIG. 7. Exosmosis of electrolytes from potato in solutions of normal propyl alcohol of various concentrations.

FIG. 8. Exosmosis of electrolytes from potato in solutions of isobutyl alcohol of various concentrations.

pected from those already cited. The results obtained are shown graphically in the accompanying figures. By comparison of the various curves it will be noticed that the action on the permeability of the cell increases with increasing complexity of the molecule. Thus, with the octyl alcohol used, a solution of concentration of only 0.008 m. has a very rapid action in increasing the permeability of the cell.

It might be urged that in the case, for instance, of isobutyl alcohol, concentrations below 0.3 m. produce an exosmosis not differing greatly during the first 24 hours from that produced by distilled water, while solutions above that concentration produce considerably greater exosmosis, and that

FIG. 9. Exosmosis of electrolytes from potato in solutions of isoamyl alcohol of various concentrations.

FIG. 10. Exosmosis of electrolytes from potato in solutions of a secondary octyl alcohol of various concentrations.

therefore 0.3 m. is the critical concentration. But there is no reason why the exosmosis during 24 hours should be chosen rather than that during any other time. If 48 hours were chosen, a different critical value would be found; and if a shorter time, as for example 4 hours, were selected, a value

of about 0.4 m. would be obtained; and if 2 hours were chosen it would be 0.5 m.

But even if it were possible to find a critical value for the concentration in this way the values so obtained for different alcohols would not support Czapek's surface-tension theory. Thus, if 0.3 m. is regarded as the critical concentration of isobutyl alcohol required to produce exosmosis of electrolytes, we obtain by comparison with other curves the following values for the various alcohols employed. The values of the surface tension of these solutions are taken from Czapek's paper.

<i>Alcohol.</i>	<i>Concentration.</i>	<i>Surface tension.</i>
Methyl	2.5 m.	0.79
Ethyl	1.4 m.	0.74
Normal propyl	0.65 m.	0.66
Isobutyl	0.3 m.	0.60
Isoamyl	0.09 m.	0.59
Secondary octyl	0.003 m.	no data

Thus a greater lowering of the surface tension in the case of the higher alcohols as compared with the lower is required to produce the same effect on the permeability of the cell.

II. *Chloroform, Chloral Hydrate, Ether, Urethane.*

Czapek himself admits that chloroform and chloral hydrate do not follow his surface-tension rule, and he therefore attributes to them a specific

FIG. II. Exosmosis of electrolytes from potato in solutions of chloral hydrate of various concentrations.

toxic action. Ether, on the other hand, is found to follow the rule. It is difficult to understand what is meant by a specific toxic action, and why, if chloroform and chloral hydrate have a specific toxic action, ether, or for that matter any other substance, should not also have a specific toxic action.

Fig. 11 shows the relation between time and exosmosis for a variety of concentrations of chloral hydrate. It will be observed that the general course of events is similar to that in the case of the alcohols, and that here also it is impossible to find a definite critical concentration above which exosmosis takes place and below which it does not. Chloroform, ether, and urethane give similar results, and it is unnecessary to reproduce the curves here. According to Czapek the surface tension of 0.1 m. chloral hydrate solution is about 0.96. There is no question of the surface-tension rule holding in this case.

As regards Czapek's theory of a specific toxic action in chloral hydrate and chloroform which does not exist in the case of the monohydric alcohols, such a view simply resolves itself into the assumption that in the case of the monohydric alcohols surface tension is the essential factor in producing exosmosis, in the case of chloral hydrate and chloroform it is some other factor. The theory that exosmosis is dependent on one physical factor, and one only, actually breaks down under the test of its author's own observations. We shall discuss this matter more fully in the theoretical part of this paper.

III. Acetone.

Experiments with acetone produced results similar to those already recorded for other organic substances. In the case of acetone more than in

FIG. 12. Exosmosis of electrolytes from potato in solutions of acetone of various concentrations.

any other might it be suggested that there exists a critical concentration with regard to exosmosis between m. and 1.5 m. (cf. Fig. 12). But it will be observed that even here exosmosis continually rises in the lower strengths of solution, and in 0.5 m., the lowest concentration examined, the exosmosis

risks continually to its maximum value. The surface tension of this solution, according to Czapek, is 0.86, so that even if there were such a thing as a critical concentration it would be lower than 0.5 m., and its surface tension higher than 0.86. Specific toxic action might again be invoked to explain this deviation from the 0.68 rule, but, according to Czapek, acetone obeys the law.

IV. Aniline and pyridine.

These two aromatic substances are both basic, and have the same surface tension in contact with air. The extent to which their solutions increase

FIG. 13. Exosmosis of electrolytes from potato in solutions of aniline of various concentrations.

FIG. 14. Exosmosis of electrolytes from potato in solutions of pyridine of various concentrations.

the permeability of potato cells to electrolytes is shown diagrammatically in Figs. 13 and 14. It will be observed that the curves are similar to those obtained with other organic substances examined, but that, in spite of the equality of the surface tensions of the two substances, aniline is considerably more efficient in bringing about exosmosis than pyridine.

No doubt here again Czapek would refer to the specific action of these basic substances, but that of course is only another way of stating that the surface tension is not the deciding factor. It may be mentioned that with both these substances the external liquid becomes coloured a bright yellow colour when exosmosis is complete, as contrasted with the dull pale brown colour produced by the monohydric alcohols.

A GENERAL CRITICISM OF CZAPEK'S THEORY OF THE PLASMA MEMBRANE.

The criticisms to which Czapek's work has been subjected by earlier writers deal chiefly with his theoretical considerations. In this contribution to the subject we have dealt mainly with the trustworthiness of the experimental evidence on which those conclusions are based. The steps, both experimental and theoretical, which lead up to Czapek's theory of the plasma membrane are these:

1. The measurement of the concentration of a solution of a substance required to bring about exosmosis from plant tissue.
2. The conclusion that there is a critical concentration below which exosmosis from the cell does not take place.
3. The measurement of the surface tension of the solutions used.
4. The conclusion that the surface tension of the solution of critical concentration against air is 0.68, that of pure water being taken as unity.
5. The conclusion that therefore the surface tension of the outer layer of the plasma against air is also 0.68.
6. The measurement of the surface tension of emulsions of neutral fats shows that their surface tension cannot be lowered below 0.68.
7. The conclusion from this that the substances in the outer layer of the plasma important in permeability phenomena are the neutral fats.

We propose now to consider each one of these steps in detail.

1. We have shown in the part of this paper dealing with methods that Czapek's method is crude and cannot be used for following changes quantitatively. By its use only very approximate results are to be expected. Sections of leaves are left in the solutions until an equilibrium condition has been reached. There is no definite time allowed for equilibrium to be obtained, the only criterion for equilibrium being apparently that the desired result is reached. Thus, in the case of tertiary butyl alcohol Czapek required a solution of 3 per cent. by volume to produce exosmosis of tannin

from *Echeveria* leaves. After one day, however, this had not happened, so the experiment was left for three days, when it had.

On the other hand, certain anthocyan-containing objects were not used because they produced exosmosis in less than twenty-four hours from diluter solutions than they should have done according to Czapek's theory. The long time required to produce the desired result in the case of *Echeveria* leaves in tertiary butyl alcohol Czapek explains as due to the slow rate of diffusion of the alcohol; the rapid exosmosis produced from various tissues containing anthocyan is attributed by Czapek to secondary injury.

It is obvious that with such methods of inquiry any desired result can be obtained, and even for these reasons alone it seems to us that Czapek's results are open to grave suspicion.

2. The results of our own experiments described in this paper indicate that there is no critical concentration below which no exosmosis of cell contents takes place. In our chief experiments we have measured the exosmosis of electrolytes, which can be done with much more accuracy than that of anthocyan or tannin. It is clear that after any time exosmosis above a certain value will have resulted in some solutions and not in others, and it might be supposed that a solution which just produced a certain quantity of exosmosis in a certain time had the critical concentration for exosmosis. This concentration would, however, vary with time chosen.

It may be noted here that Koltzoff (6) has already criticized Czapek for neglecting the time of reaction in his experiments. To this Czapek has replied (4) that he deals only with equilibrium conditions and has therefore eliminated the factor. Our experiments show, however, that Czapek could only have had equilibrium conditions in those solutions in which exosmosis had occurred, and that exosmosis took place, if slowly, in those where it was not measurable by Czapek's imperfect method. The time factor is there; it cannot be eliminated merely by neglecting it. From these considerations we do not accept Czapek's supposition that there is a definite critical concentration required to produce exosmosis.

3. Koltzoff (6) and Vernon (11) have already called attention to the fact that Czapek measures the surface tension of his solutions against air, whereas what he is really concerned with in his experiments is the surface tension between the solutions and the plasma. Czapek assumes that the lowering of the surface tension of a liquid in contact with air, due to dissolved substances, runs parallel to the lowering of the tension when in contact with another liquid. Vernon quotes Clerk Maxwell, who states that by no known means can the surface tension between two immiscible liquids be determined from their surface tensions in contact with air. Thus, the relation between Czapek's surface-tension measurements and the surface tensions actually existing in his experiments is unknown, and hence it is

impossible to draw general conclusions from such measurements. To this criticism Czapek has made no satisfactory reply.

4. Czapek shows that seven substances out of twenty-nine examined by him have a surface tension far greater than 0.68. This has also been pointed out by Vernon. To this criticism Czapek (4) replies that he has already explained the deviation of these substances from the 0.68 rule as being due to their 'specific toxic action', although this is absolutely nothing more than stating the same fact in different words. Vernon suggests that in the cases of these exceptions, the substances probably dissolved the lipid substances in the membrane, and that it is to be explained on the Meyer-Overton law of partition coefficients. Vernon points out that the same order of concentrations is obtained for tannin exosmosis as for narcosis, in which the results are explicable as due to lipid substances.

But Schryver (8) suggests that there is no need to assume the results for narcotics as due to lipoids. This author shows that if substances are arranged in the order of their power of disintegrating a cholate gel, that order is exactly the same as found by Czapek in regard to tannin exosmosis, and as Schryver says, the cholate gel 'cannot, by any extension of the meaning of the term, be described as a lipid', and in any case Czapek's 'purely mechanical conception of cytolysis is clearly no longer tenable'.¹

In any case Czapek's assumption of specific toxic action is really most destructive of his whole case, for it is an admission that factors other than surface tension may in some cases be the predominating ones in regard to exosmosis.

Again, it cannot even be conceded that solutions which produce equal exosmotic effects have the same surface tension in contact with air. The following table is compiled from the results recorded in this paper. The values are only approximate, but they are at any rate considerably more trustworthy than those of Czapek.

<i>Substance.</i>	<i>Strength required to produce same exosmotic effect.</i>	<i>Surface tension against air (from the data of Czapek and Traube).</i>
Methyl alcohol	3.2 m.	0.76
Ethyl ,,	1.6 m.	0.70
Normal propyl alcohol	0.7 m.	0.64
Isobutyl ,,	0.33 m.	0.58
Isoamyl ,,	0.095 m.	0.58
Secondary octyl ,,	0.0048 m.	no data
Chloral hydrate	0.17 m.	0.94
Urethane	0.5 m.	0.77
Acetone	1.3 m.	0.69
Aniline	0.08 m.	no data
Pyridine	0.45 m.	„

¹ On the other hand, we do not regard Schryver's experiments as having any direct bearing on cell problems. The comparison of the cholate gel with the cell rests on the similarity of the power of certain substances to disintegrate the gel, and their power of producing exosmosis from the cell.

It is obvious from these numbers that the exosmosis of electrolytes from the cell, and consequently the permeability changes produced by them, cannot be referred to surface tension alone.

The rate at which the external liquid displaces gases in the intercellular space system may be of importance in this connexion. It is a matter of common knowledge that alcohols displace the air in the intercellular spaces very rapidly, while aqueous solutions do so slowly. Hence cytologists in fixing plant material with aqueous fixatives pump out the air from the tissue in order that the fixing fluid may be brought in contact with the cell membranes bordering on the intercellular spaces. In Czapek's experiments and our own the same considerations hold. The lower the surface tension against air, the easier is the air displaced from the intercellular spaces (cf. Czapek's method of measuring surface tension). Hence in the lower strengths of solution used the rate of exosmosis may be lower than it would be if the air were displaced rapidly as in the higher concentrations of solution. However, with thin sections of tissue the difference is not likely to be very great.

5. The assumption is made that if solutions whose surface tension in contact with air is 0.68 are just strong enough to produce exosmosis from the cell, therefore the surface tension of the outer layer of the protoplasm towards air must also be 0.68. As far as we are aware there is not a shred of evidence in support of this assumption. In his later paper (4) Czapek explains this by supposing that at the interface the substance which most lowers the surface tension will displace those which lower it less. Even if this statement is true, it appears to have no bearing whatever on the problem in hand. For methyl alcohol, for instance, lowers surface tension less than ethyl alcohol, and according to Czapek's assumption ethyl alcohol, if it were present in the plasma membrane, would not be replaced by methyl alcohol in whatever concentration, while if ethyl alcohol were outside and methyl alcohol inside the plasma this would be replaced by ethyl alcohol even if it were below the critical concentration. In short, it would be impossible to accept this step in Czapek's theory of the plasma membrane, even if the previous steps in the argument could be accepted.

6. It is difficult to understand what Czapek means when he asserts that emulsions of neutral fats in water will not lower the surface tension of water below 0.68, as he himself quotes several examples of fats, emulsions of which with water lower the surface tension very considerably below this value.

7. Finally, even if we could accept the evidence that the surface tension of the plasma membrane in contact with air is 0.68, and if it were a fact that fat emulsions cannot lower the surface tension of water in contact with air below 0.68, this is no reason for concluding that the essential substances in the protoplasm as regards its surface tension are neutral fats. For as Czapek

himself admits, substances which could lower the surface tension more than this might be present in the plasma membrane in diluter solution. Thus, for example, he says : 'Meine Beobachtungen widerstreiten nun nicht direkt der Annahme, dass verdünnte Emulsionen von Lezithin und Cholesterin das eigentümliche Verhalten der Plasmahaut bedingen, doch bringen sie auch keinen weiteren Grund dafür bei, dass die Annahme Overtons die allein richtige sein könne.' Similarly, this writer also admits that proteins may be present in the plasma membrane.

From this review of the details of Czapek's work on the plasma membrane, it is clear that neither the experimental evidence nor any part of the theory based upon it can be accepted.

THE APPLICATION OF THE LAWS OF MASS ACTION TO THE QUESTION OF EXOSMOSIS FROM PLANT TISSUE IN THE CASE OF IRREVERSIBLE CHANGES OF PERMEABILITY.

When plant tissue is immersed in a solution of a substance we may conclude that after a certain time, owing to diffusion through the intercellular spaces of the tissue and through the cell-walls, a large part of the outer layer of the protoplasm of the cells will be in contact with the solution. If the solute acts either physically or chemically with one or more substances in the protoplasm, the rate at which it does this will depend upon the concentration of the solute at the limiting surface, and also upon the concentration at the limiting surface of the substance with which it reacts. Other factors, such as temperature for instance, may also influence the rate of reaction. The concentration of the reacting substances at the interface between the two phases will depend upon the extent to which the surface tension is lowered according to the principle of Willard Gibbs, but this is only one of a number of factors which may affect the concentration at the interface. Schryver (7) has pointed out that in an action resembling somewhat the one under consideration, namely that of the disaggregating action of salt solutions on globulins, the rate of diffusion of the substances in solution must also influence the rate of reaction. For if diffusion is slow the accumulation of solute in the surface layer may fall below that given by the Willard Gibbs rule, owing to the removal of the substance in the action at the interface at a quicker rate than it can be replaced by diffusion of solute from other parts of the solution. As the rate of diffusion will depend on the viscosity, Schryver concludes that 'the disaggregating action of salt solutions on the globulins is a function of two physical constants of the solutions, viz. the viscosities and the surface tensions'. But of course it is understood that the salt solution has an action on the globulin. The surface tensions and viscosities influence the rate of the reaction; they do not decide whether an action takes place or not. Other factors may come into

play also; thus the effect of electric charges at the surface in influencing the concentration there of the reacting substances cannot be neglected.

While we may conclude that the rate of the action increasing the permeability of the cell is dependent on the concentration of the active substance at the surface of the cell protoplasm, we cannot say what function of the concentration the rate of reaction is. If the action were a purely chemical one we could say from the law of mass action that the rate of reaction is directly proportional to the active mass of the substance in the limiting layer. But besides chemical actions others are possible which might alter the state of aggregation of substances in the protoplasm—by solution, for instance, or by precipitation of a substance from a colloidal solution. In such cases the disaggregation effect is not simply proportional to the concentration of the solution. A reference to the solution or precipitation of certain proteins such as gliadin by alcohol is sufficient to show this, and we may mention Schryver's work (9) on the cholate gel in the same connexion, in which he shows a most curious relation existing between concentration of solutions and their rate of disaggregation of the gel.

Moreover, as far as we know, any substances present in the protoplasm are also present in the limiting layer, and it is quite possible that different substances in the plasma may be affected in different ways by the same solute.

As it is doubtful what relation the concentration of the solute bears to its concentration at the interface, and as it seems likely that this latter quantity will vary at different parts of the tissue owing to variation in rates of diffusion connected with differences in position of cells in different parts of the tissue, it is difficult to draw conclusions as to what function of the concentration of the solution is the change of permeability produced by it.

If, however, we consider a case in which the concentration is so high that the quantity of solute used up is negligible, and if we make the assumption that the concentration at the surface of the plasma membrane remains constant throughout the reaction time, we can from the ordinary laws of mass action obtain an equation connecting the exosmosis with time, and the curve representing this equation is of the same form as those obtained in the experiments described in this paper.

For we may suppose that the increase in permeability of the cell is due to a change in a substance present in the outer layer of the protoplast, whose active mass before the experiment is M . Let us suppose that at any time t of the total amount M , a part m has been destroyed.

The rate at which further destruction of the substances takes place will depend on the quantity left, $M - m$, and on the concentration of the external solution.

Hence

$$\frac{dm}{dt} = \alpha f(M-m) \phi(C),$$

where $f(M-m)$ is a function of the mass of substance left in the membrane unchanged and $\phi(C)$ is a function of the concentration of the external solution, α being a constant.

If this concentration is so high that only a small quantity is used up throughout the experiment, $\phi(C)$ is a constant for any particular concentration. Also it may be assumed that the rate of change of the substance in the membrane is directly proportional to the amount of it left, when the equation therefore becomes

$$\frac{dm}{dt} = A(M-m), A \text{ being a constant,}$$

$$\text{or } \frac{dm}{M-m} = A dt;$$

whence, integrating between limits,

$$\log \frac{M-m}{M} = -At$$

$$\text{or } \frac{M-m}{M} = e^{-At}$$

$$\text{and } m = M(1 - e^{-At}).$$

Now the rate of exosmosis will be proportional to the amount of the membrane substance destroyed, and also to the difference in concentration of electrolytes inside and outside the tissue.

If at the time t the concentration of the electrolytes in the outer solution is s , and inside the cell s' , the rate of exosmosis is

$$\frac{ds}{dt} = \beta m(s' - s),$$

where β is a constant.

If S is the concentration of electrolytes inside the cell at the beginning of the experiment, and if u and v are the respective volumes of the tissue and external solution

$$u(S - s') = vs$$

$$\text{or } s' = S - \frac{vs}{u};$$

whence

$$\frac{ds}{dt} = \beta M(1 - e^{-At}) \left(S - s \frac{u+v}{u} \right)$$

and

$$\int \frac{ds}{S - s \frac{u+v}{u}} = \int \beta M(1 - e^{-At}) dt,$$

from which, integrating between limits, we have

$$-\frac{u}{u+v} \left\{ \log \frac{S-s \frac{u+v}{u}}{S} \right\} = \beta M \left\{ t + \frac{1}{A} (e^{-At} - 1) \right\},$$

which may be written in the form

$$-B \log (1 - ks) = D \left(\frac{1}{A} e^{-At} + t - \frac{1}{A} \right),$$

where s is the concentration of the external solution at any time t and A , B , D and k are constants depending on the concentration of the external solution, the quantity of tissue used, and the volume of solution.

The curves given by this equation are of the same form as those obtained by us for the exosmosis of electrolytes from plant tissue. In the accompanying figure we give the curves obtained in which the constants B ,

Fig. 15. Curves obtained from theoretical considerations of the relation between time and quantity of exosmosis from plant tissue.

D , and k are put equal to unity, and A , which depends on the concentration of the external solution, is given different values. A comparison of these curves with those for the higher concentrations of each substance experimented with will show how similar they are.¹

It will be observed that the curve consists of three regions: first a region in which the rate of exosmosis is gradually increasing, then

¹ The system is certainly more complex than we have assumed here. For instance, we have neglected the rate of diffusion of substances in the system. In thick discs and cylinders of tissues this becomes a very important factor, as some of our unpublished researches show. The equation given can only be considered a very rough approximation.

a region during which the rate remains more or less constant, and a final region in which the rate gradually gets less and less. The first part of the curve corresponds to the period during which the impermeable substance in the plasma is rapidly breaking down. The rate at which this takes place gradually gets less until the changed substance remains practically constant in amount; the difference in concentration of electrolytes inside and outside is then the chief factor in determining the rate of exosmosis. This period corresponds to the last part of the curve, in front of which we have the approximately straight transitional part.

With very high concentrations the first part of the curve is extremely short, and in practice readings have to be taken very frequently to show it, and it may be too brief to show graphically on the curves at all.

In all these considerations we have assumed that the concentration of the external solution is so high that it remains practically unchanged throughout the experiments. But if this should become appreciably less as a result of the action, the rate at which the membrane is changed will be much lessened as time goes on. Consequently the rate of exosmosis will be considerably less than it would be if the concentration of the external solution remained constant. The curve we should expect under such conditions is precisely that obtained by us for the lower concentrations of the external solutions we have employed.

SUMMARY.

1. The action of various organic substances on the permeability of plant cells has been investigated by estimating the exosmosis of electrolytes from plant tissue immersed in aqueous solutions of the substances. The exosmosis is estimated by measuring the rise in electrical conductivity of the solution in which the tissue is contained, due regard being had to the depression of conductivity resulting from the presence of the non-electrolytic experimental solutions.

2. The method is compared with various methods employed by former workers, and evidence is brought forward to show that it is more exact and of more general application than those earlier methods.

3. With each organic substance the rate of exosmosis depends on the concentration of the substance employed; the higher the concentration the more rapid the exosmosis.

4. Equimolecular solutions of different substances do not bring about the same exosmosis. Thus, in the homologous series of the monohydric alcohols, the more complex the molecule the greater the exosmosis produced by solutions of equimolecular strength.

5. The rate of exosmosis produced by a solution is not a function of its surface tension alone.

6. It is impossible to find a critical concentration of a solution below which exosmosis of electrolytes will not take place.

7. Each of the assumptions leading up to Czapek's theory of the plasma membrane is discussed, and it is shown that none of them can be accepted.

8. An attempt has been made to deduce a mathematical expression connecting time and exosmosis, and it is shown that the curve representing the equation so obtained from theoretical considerations is of an exactly similar form to those obtained in actual experiments.

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