THE PROBABLE ACTION OF LIPOIDS IN GROWTH.

By D. T. MacDOUGAL.

(*Read April 21, 1922.*)

Accumulating evidence, including biochemical tests of the occurrence of lipoids in cells, especially abundant in the accretion stage of growth, microscopic and ultra-microscopic examinations of plasma and wall, the use of reagents which would displace or liquefy lipoids in the modification of permeability, all support the conclusion that the lipoids constitute the external layer of the plasma sending a penetrating meshwork into both the plasma and the wall.

The implied view would make the lipoids the fundamental structure of protoplasm and the primary factor in all exchanges between the cell and the medium. The facts cited cause renewed interest in the original proposal of Quincke in 1888 and of Overton in 1895 and 1899 as to the lipoid theory of the plasmatic membrane.

Additional information concerning the nature and action of some of the common lipoids, and of the nature of hydration, together with exact determinations of the influence of various ions on permeability, gives opportunity for the consideration of the subject from new angles.

1. The experimental results which concern the matter and which are described in the present paper have been obtained by two methods. Measurements of endosmose in artificial cells, with a plasmatic lining including lipoids, have been made. These results have been correlated with measurements of the hydration reactions of biocolloids, and living and dried cell-masses in the solutions used in the osmotic tests.

2. Lecithin incorporated in the plasmatic jelly layer of an artificial cell has but little effect on the osmotic action. The same substance deposited as a layer between the plasmatic jelly and the outer wall lessens the permeability of the system and increases the osmotic effect.

3. The use of saponin or solutions which liquefy or displace lecithin in the cell contents or external layer increases permeability, presumably by increasing hydration, and lessens osmotic action. This is in agreement with the results of Boas and Kahho.

4. The nature of the action of saponin on artificial cells suggests that this substance affects the permeability of the outer clay walls of artificial cells. The hydration reactions of living and dead cell-masses measured with the auxograph show a similar influence on the wall of the plant cell.

5. Dried plates of biocolloids which show many similarities in hydration to the action of protoplasm, but which contain no lipoid, are rendered less permeable when swelled in a saponin solution. Such a solution increases the permeability of plant cells. The results support the inference that this effect is due to the action of the solution upon the lipoid constituent.[1]

6. The artificial cell as used in the experiments shows accelerated action when a lipoidal layer is deposited between the plasmatic jelly and the outer clay wall. With cell-contents of NaCl 0.01*M* immersed in $CaCl_2$ 0.001*M*, negative osmose, then positive osmose, occurs. The tonicity of the cell may be seen to increase from 0.003 to .005*M* KCl. The "life" or period of activity of such a cell may extend over a period of 60 to 80 days with renewal of the immersion liquid, but not of the cell-contents. Anomalous osmose may be exhibited by the outer wall, which is semi-permeable to sugar, asparagin, and other organic substances, but the action of the cell when the plasmatic layer is added is positive.

7. Living cell-masses which show a water deficit or a hydration capacity of 40 per cent. and which remain turgid when swollen take up only about one fourth of this amount of water when increased permeability sets up shrinkage in saponin 0.005*M*. The effect of the saponin decreases with the concentration to a minimum at about 0.0002*M*.

8. The effect of the saponin upon permeability as measured by the swelling which ensues before shrinkage begins is not definitely accel-

[1] Quincke, G., *Ann. d. Physik u. Chemie*, N. F., 35, 580, 1888. Overton, E., "Osmotische Eigenschaften," *Vierteljahrsschr. Naturforsch. Ges.*, Zurich, 40, 1, 1895. See also "Ueber die allegemeinen osmotischen Eigenschaften d. Zelle" by this author in the same publication, 44, 110, 1899.

erated by KCl in living cell-masses of *Opuntia*. This result is not in agreement with Boas, who found that the salt accelerated the action of saponin upon cells of *Tradescantia*.

9. The hydration of such living cell-masses is not definitely affected by variations in acidity up to PH 2. No positive increases were noted in KHO in weaker concentrations, but swelling was lessened in 0.01*N*.

10. Dried cell-masses display a minimum effect from saponin at about the same concentration as the living material, but the swelling which is maintained increases with the concentration to about 0.005*M*.

11. The hydration of dead cell-masses was not definitely affected by HCl at 0.01*N*, but at weaker concentrations, 0.001 to 0.0002*N*, the swelling was greater than in water. After the neutral point is passed some increase is to be noted in KHO 0.001*N*, which reaches its maximum at 0.01*N*.

12. The hydration reactions of dead cell-masses of *Opuntia* in acidified solutions are those which might be displayed by a biocolloid in which the protein and pentosan components were nearly equal in quantity. The hydration reactions of living cell-masses are such that permeability is increased and swelling lessened in hydroxide at 0.01*N* and 0.005*N*. This reaction, like that of saponin, is reversed in the dead cell-masses in which swelling increases with the concentration.

Cytologists have for the most part considered the so-called plasmatic membrane as a peripheral layer not separable from the cytoplasm and by implication to consist of proteins. Seifriz regards this suppositious membrane as a highly viscous layer about 1 μ in thickness.[2] In the recent notable contribution by Hansteen-Cranner the peripheral layer of plasmatic substance is regarded as lipoidal, consisting of a disperse phase of hydratable material not soluble in water, in a continuous phase of water-soluble lipoid. This formation is continuous with a fundamental lipoid meshwork of the plasma. A lipoid meshwork from the peripheral layer is also supposed to extend through or into the cell-walls. The conclusions thus briefly noted are held to sustain the general contention of Overton as to the lipoidal

[2] Seifriz, W., "Observations on Some Physical Properties of Protoplasm by Aid of Microdissection," *Annals of Bot.*, 35: 269–296, 1921.

character of the plasmatic membrane.[3] Such an arrangement of lipoids would be one which would permit the passage of both water-soluble and fat-soluble substances into the cell.

Czapek's notable contributions to this subject show that lipoids are especially abundant in meristem and in nearly all cells in the accretion stage of growth. Czapek places himself in the position of considering the plasma as essentially a lipoidal structure.[4] Walter, in confirmation of Biedermann, found that the plasmatic mass of plants is not readily digested by proteoclastic enzymes until the lipoid, which is held to be in a fine state of dispersion, is first extracted by a fat solvent. He holds that his evidence is against the conclusion that the lipoids are localized in a peripheral layer in the cell.[5]

The presence of lipoids in the cell colloids in the accretion stage of growth would, it seems, almost inevitably result in their accumulation in the peripheral portion of the plasmatic mass in accordance with the laws of surface tension. That such a layer does exist was concluded by Boas, who published a preliminary paper to this effect in 1920 and his detailed observations upon which this conclusion was reached in 1921. The experiments were based upon the known reactions of lecithin and cholesterin to neutral salts and to saponin. Measurement of fermentation in yeasts and of the decolorization of cells of higher plants are made the basis for the assertion that a proteinaceous membrane in the cell is highly improbable, and that lipoids are concerned in the exchanges of the cell with the medium.[6] Although this writer found that non-conductors in hypotonic solution retarded the action of saponin on the supposed lipoidal layer, and that hypertonic solutions of cane sugar, for example, accelerated it, the

[3] Hansteen-Cranner, B., "Beiträge zur Chemie und Physiologie der Zellwand und der plasmatischen Grenzschichten," *Ber. d. Deut. Bot. Gesell.*, 37, Hft. 8, 380–391, 1919.

[4] Czapek, F., "Zum Nachweise von Lipoiden in Pflanzenzellen," *Ber. d. Deut. Bot. Gesell.*, 37: 207–216, 1919.

[5] Walter, H., "Ein Beitrag zur Frage der chemischen Konstitution der Protoplasma," *Biochem. Zeit.*, 122: 86–99, 1921.

[6] Boas, Fr., "Beitrage zur Kenntniss der Wirkung des Saponins auf die Pflanzliche Zelle," *Ber. d. Deut. Bot. Gesell.*, 38: 350–353, 1920. Also, "Untersuchungen ueber die Mitwirkung der Lipoide beim Stoff-Austausch der Pflanzlichen Zell," *Biochem. Zeit.*, 117: 166–214, 1921.

possibility of the action on other colloids than proteins and lipoids is not considered. Kahho published the results of his tests as to the permeability of roots of yellow lupine to neutral salts late in 1921. He confirms the series as to permeability of kations which runs K>Na>Li>Mg>Ba>Ca, in which the greatest penetrability is shown by potassium and the least by calcium. It is also seen that the interferences are such that each kation is retarded by those to its right, and to a degree proportionate to its distance to the right, and that the greatest retardation is by the kations which show the greatest coagulating action on colloids. The kations which have the least coagulating action on colloids penetrate most rapidly. The anions retard the colloidal or coagulative action of the kations in a series, citrate<sulphate<tartrate<Cl<NO_3<Br<I, in which the effect is least with the citrate and most with the iodine. That is, each kation has the greatest effect when combined with the citrate and least with the iodine. As a further consequence, the citrates have the least penetrability and the salts of iodine greatest. It is held that the behavior of the roots in a weak alkaline solution supports the conclusion of Hansteen-Cranner as to a lipoidal layer.[7] The greater expansion and contraction of growing roots in weak alkaline solutions is attributed to the solution or displacement action of such solutions on the lipoidal meshwork of the walls, rendering them more contractile.

The results cited above are not decisive or final when taken separately. Their concurrence lends substantial support to the contention that the lipoids are a prime factor in the exchanges between the cell and the medium. The establishment of the fact that such a lipoid as lecithin may be present as an emulsion consisting of a disperse medium soluble in water and of a disperse phase swelling in water, the entire system displaceable by fat-solvents, would furnish a plasmatic membrane or peripheral layer through which both salts and fatty substances might diffuse. Lecithin, for example, is supposed to absorb about 40 per cent. of its volume in hydration in water, is soluble in chloroform, alcohol, benzene, carbon disulphide, etc., and has the power of combining with both acids and bases.

[7] Kahho, H., "Ein Beitrag zur Permeabilitat des Pflanzenprotoplasmas für Neutralsalze," *Biorhem. Zeit.*, 120: 284–303, 1921. See also p. 125, same volume.

The importance of the entire matter is such that two series of experiments were designed in my own laboratory, the results of which might have a bearing upon the above conclusions. In one the auxograph was used to register variations in thickness indicative of changes in turgidity of cell-masses subjected to neutral salts and other solutions. Next lipoids were introduced into the construction of the artificial cell recently designed, and the effect of such substances upon permeability of plasmatic layers of cell colloids under the influence of salts, saponin, and soaps was determined.

Brief mention has already been made of the artificial cell used.[8] The cell in question was of a design in which clay, porcelain, alundum, or wooden thimbles representing various degrees of porosity were used to represent the external wall, while the plasmatic layer could be represented by a plasmatic lining layer of any jelly or mixture of jellies. The thimbles were fitted with an osmometer head consisting of a stopper pierced with two holes, in one of which was fitted a filling funnel with stopcock, and the other with an outlet tube bent to the horizontal immediately above the stopper. Such an arrangement permitted the measurement of endosmosis by the amount of liquid forced out and caught in a small graduated receiver. The greater number of experiments were made with the clay thimbles used in the Livingston evaporimeter (Fig. 1). Cells of this type lined with agar treated with tanning reagents and fitted with vertical outlets to show pressure, designed by Professor H. M. Richards, have been in use for some time in the Botanical Laboratories of Barnard College. As arranged in the work described here, the pressures in the cell were never more than that of 12 or 15 mm. of water.

The arrangement of this artificial cell was begun by washing the thimbles in warm distilled water and preparing a liquid mixture of the materials for the plasmatic lining layer. About 10–12 cc. of this material was poured into the warm, moist thimble at a temperature of 40–50° C., the osmometer head put in place, and the thimble turned in the hand in a horizontal position. If a coating of 2 or 3 mm. in

[8] MacDougal, D. T., "The Distentive Agencies in the Growth of the Cell," *Proc. Soc. Exper. Biol. and Med.*, 19: 103–110, 1921. See original description of this cell in the *Report of the Dept. of Bot. Res., Carnegie Inst. Wash.* for 1921.

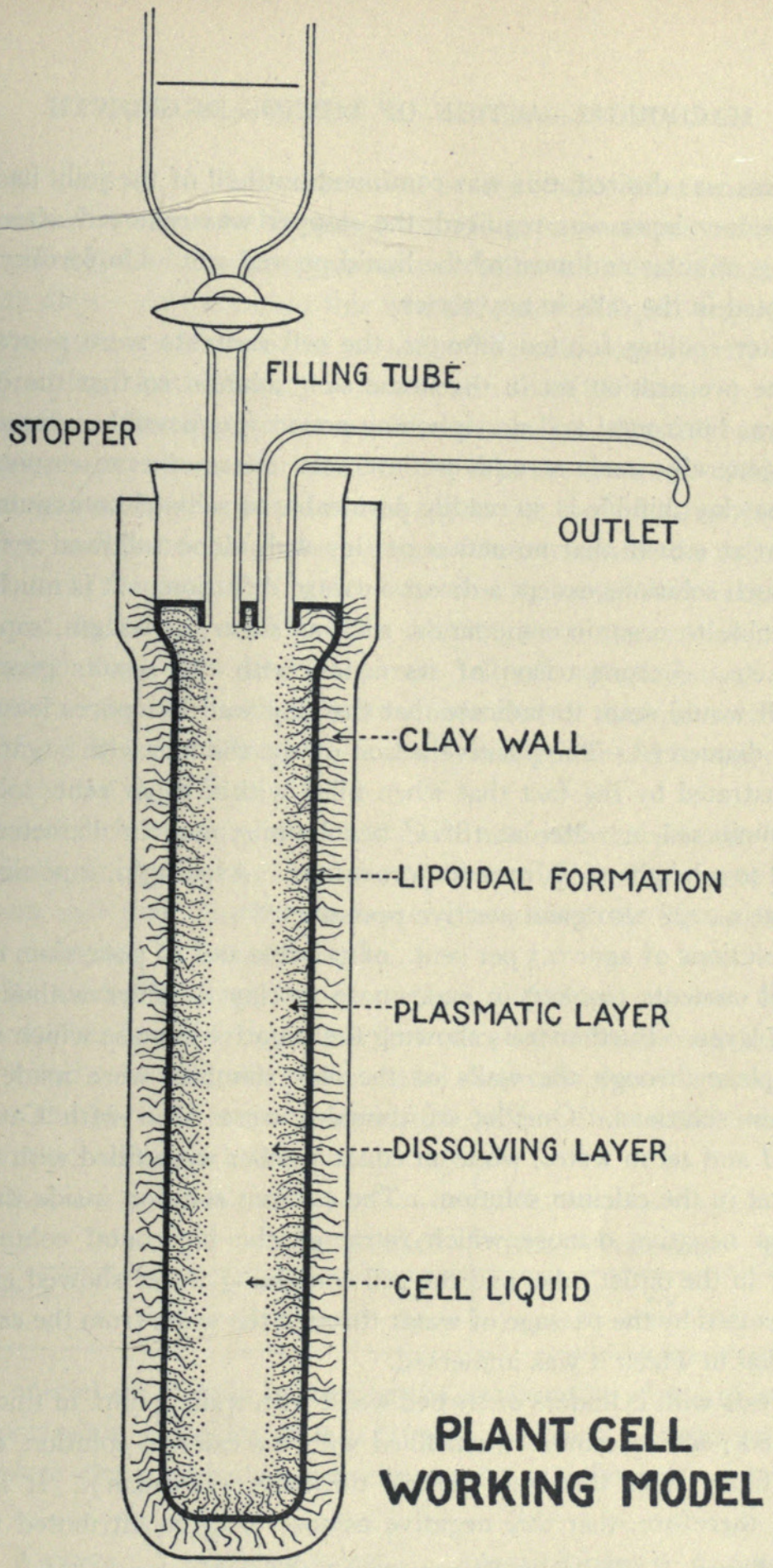

FIG. 1. Artificial cell designed to illustrate variations in outer wall and plasmatic layer. The clay wall is that of a filter thimble such as is used in the Livingston evaporimeter. The lipoidal layer is drawn heavily out of proportion to illustrate more clearly the processes extending into the wall and the plasmatic layer. The plasmatic layer in the experiments described in this paper was composed of agar, agar-gelatine, or modified mixtures. The cell was submerged to the top of the stopper in operation.

thickness was desired, this was continued until all of the jelly had set. If a thinner layer was required, the stopper was removed after two or three minutes and most of the liquid poured out. Uniformity was attempted in the cells in any series.

After cooling for ten minutes, the cell contents were poured in and the preparation set in the immersion solution so that the outlet tube was horizontal and no siphoning action was possible. Readings were generally made at 24-hour intervals unless otherwise specified.

The clay thimble is so readily permeable to salts of potassium and sodium at 0.01*M* that no action of this wall alone followed a filling with such solutions except a direct outward diffusion. It is much less permeable to organic compounds, such as sugar, asparagin, saponin, soap, etc. A comparison of its action with the results given by Bartell would seem to indicate that the clay wall has pores less than 1 μ in diameter.[9] The positive action of the thimble with sugar may be illustrated by the fact that when filled with a 2 per cent. solution and immersed in water at 18° C. a column 3 mm. in diameter was raised to a height of 35 mm. in 30 minutes. Asparagin, saponin, and urea at 0.01*M* also gave positive pressures.[10]

Solutions of agar 0.3 per cent., of gelatine and of potassium oleate as cell contents resulted in endosmose in clay thimbles without any lining layer. Further tests showing the negative osmose which might take place through the walls of the clay thimbles were made with calcium solutions. One lot of thimbles were filled with $CaCl_2$ at 0.01*M* and set in water, while an equal number were filled with water and set in the calcium solution. The calcium solution inside the cell set up negative osmose which retracted the horizontal column of water in the outlet tube, and the cell containing water showed exudation caused by the passage of water through the walls from the calcium solution in which it was immersed.

Tests with cylinders of turned wood with walls 3 mm. in thickness showed positive osmose when filled with the calcium solution, as did also filter paper thimbles (double thickness Whatman). It is evident, therefore, that this negative osmose is to be attributed to the

[9] Bartell, F. E., "Pore Diameters of Osmotic Membranes," *Jour. Phys. Chem.*, 16: 318, No. 4, April, 1912.

[10] Stern, K., "Ueber negativen osmosen und verwandten Erscheinungen," *Ber. d. Deut. Bot. Ges.*, 37: 334–343, 1919.

size of the pore or the composition of the wall. The repetition of the tests with calcium chloride at 0.001*M* as an immersion fluid on a clay cell filled with water gave an exudation of 1.3 to 1.6 daily for twenty days. At the end of this time the external liquid was replaced by one more concentrated at 0.01*M*. The rate rose slightly during the first day, then fell away to 0.2 c.c. on the fifth day.

Another interesting case was that in which a clay cell containing sodium at 0.01*M* was set in a calcium solution 0.001*M*. The initial action was a negative osmose resulting in the loss of water from the stronger sodium to the weaker calcium solution outside the cell during the first 24 hours. The action was now reversed and endosmosis began at the rate of 0.2 c.c. daily, which rose to 1.4 c.c. daily on the fourteenth day, after which the rate fell off. Replacement of the immersion solution with a more concentrated calcium solution at 0.01*M* did not alter this process, as the rate continued to decrease to 0.2 c.c. on the tenth day.

After such measurements had been made of the porosity of the clay walls, lining layers of various mixtures of biocolloids were placed in them and their absorption capacity measured by the amount of water delivered by the outlet tube.

The examples given below are sets selected at random from a large number in which no radical departure from the average behavior was found. Unless otherwise stated the cells were immersed in water, and the cell contents were at 0.01*M*. Endosmose measured as excretion is given below in c.c.

	KCl.	NaCl.	HCl.	Asparagin.	Water.
Gelatine	2.4	2.6	1.6	2.9	1.5
Agar	4.5	3.6	5.3	3.1	1.6

A special set of cells for testing the comparative effects of sodium and potassium chlorides at 0.01*M* with a plasma of agar-gelatine-potassium oleate was operated with results as follows:

Contents.	Outside Liquid.	Amounts Exuded.	Average.
Water	Water	2.2, 2.5, 2.8 c.c.	2.5 c.c.
KCl 0.01*M*	"	3.2, 6.9	4.9 "
NaCl 0.01*M*	"	2.7, 2.3	2.5 "

Repetition of these experiments gave no data varying notably from the above. All were carried out at 15° C.

A fourth plasmatic layer was prepared consisting of agar 5 g., gelatine 2 g., potassium oleate 5 mg., and lecithin 25 mg., and parallel series in potassium and sodium chloride were run at laboratory temperatures of 14 to 18° C. with results as follows, in which the total excretions for three cups with each salt are given:

KCl 0.01*M*	....5.8 c.c.	8.4 c.c.	9.4 c.c.	Total in 140 hours	23.2 c.c.
NaCl 0.01*M*	...5.2 c.c.	6.7 c.c.	8.2 c.c.	" " " "	20.1 c.c.

It is to be seen that the action of the sodium and potassium salts is most nearly equivalent in gelatine, that the greatest differences are shown in a layer of agar, and agar-gelatine soap where the amount of excretion from a potassium cell may be double that of one filled with sodium. However, when lecithin is incorporated in the mixture, the difference between the action of the two bases is not very great.

The several processes which are integrated in these results may be simply stated as follows: First, the plasmatic layers begin to go into solution as soon as the cell is filled and the resulting osmotic action will be one characteristic of such solution. About 10 per cent. of an agar-gelatine soap layer 2 or 3 mm. in thickness would be dissolved at the end of the fourth day, but after this saturation point of the cell contents is reached the plasmatic layer dissolves slowly as new supplies of water are absorbed so that cells have been seen to operate for 60 to 80 days.

It has been pointed out previously that such a cell with a plasmatic layer of agar-gelatine-potassium oleate mixture has at the beginning a tonicity by which it shows endosmosis in a 0.03*M* solution of potassium chloride. After some action in this concentration it may be moved by stages to a solution of 0.005*M* from which it will absorb water.[11]

[11] MacDougal, D. T., "Distentive Agencies in Growth," *Proc. Soc. for Exper. Biol. and Med.*, 18: 103–110, 1921.

[12] See Fitting, H., "Untersuchungen ueber die Aufnahme von Salzen in die lebende Zelle," *Jahrb. f. Wiss. Bot.*, 56: 1–64, 1915.

Stiles, M. A., and Kidd, F., "The Comparative Rate of Absorption of Various Salts by Plant Tissue," *Proc. Roy. Soc. Lond.*, B, 90, 487–504, 1919.

Kahho, H., "Ein Beitrag znr Permeabilität des Pflanzenplasmas für die Neutralsalze," *Biochem. Zeit.*, 120, 284–303, 1921.

The above applies to cells filled with water and immersed in water. When the contents include substances already in solution, such as the salts of potassium and sodium, the action of these salts on the colloids both as to their solution and hydration are to be taken into account. As hydroxide at 0.01*M* potassium is found to retard the hydration of agar more than sodium. Whether this would be true of the designated colloidal mixture, and as a chloride, has not been tested, but probably this action would be reversed.[12]

The ionic velocity and speed of penetration of the potassium would be greater, and its superior osmotic action would result in a greater endosmosis than in the cell containing sodium.

That solution of colloids, the penetration of the pores by colloidal matter, and the action of salts and other compounds act progressively may well be inferred from the long-continued swelling action of these biocolloids in dilute salt solutions and amino-compounds, on the one hand, and on the other by the fact that when biocolloidal cells are set in action the rate of endosmosis rises until some time in the third 24-hour period, no change having been made in the preparation meanwhile. A decline in the rate takes place during the fourth day, but the decrease is so slow that endosmosis may continue for 60 to 80 days. Such action is illustrated by the following figures:

Contents.	Amount of Endosmosis.					
	1st Day.	2d Day.	3d Day.	4th Day.	Re-filled 5th Day.	6th Day.
Water.....	0.3	1.5	2.8	1.7		
Urea 0.01*M*.....	0.7	1.8	3.5	2.2	2.6	2.6 c.c.
Asparagin 0.01*M*.....	0.8	2.4	4.5	3.0	1.6	2.2

The above procedure, it should be noted, is one that characterizes the agar-gelatine-soap biocolloid and is not shown by agar in which endosmosis falls off rapidly.

The foregoing tests serve to illustrate the operation of the artificial cell which it was proposed to use in determining the possible action of a peripheral layer of lecithin or lipoids in the absorption of salts by the plasmatic layer. Lecithin had been incorporated with

the other main components of a lining layer in one series of preparations, but the effects of its action as minute globules distributed throughout the mass were indeterminate, as noted above. A special series prepared in such manner as to secure a lipoidal layer external to the plasmatic mass and lying next to and in the wall was next prepared.

Clay filter thimbles which had been cleaned and warmed in distilled water were drained a few seconds, then 4 c.c. of a 2 per cent. solution or emulsion of lecithin was poured into it. After stoppering the thimble was turned in the hand for about two minutes in such manner that the lecithin was made to bathe the entire inner surface repeatedly. About 3 c.c. of liquid would be poured out when the stopper was removed, but this would be of a lighter hue indicative of the fact that some of the lipoid had been taken up by the cup, where it would be held in the inner surface layer. If the properties of the lecithin layer alone were to be tested, the osmometer head would be put in, the desired solutions poured in through the funnel tube, and the thimble set in the immersion fluid. If a more complete simulation of the conditions in the cell were desired, the thimble treated with lecithin received about 10 c.c. of liquid jelly, and after being again stoppered was turned for another two minutes, when this was also poured out, leaving a thin layer on the wall, and the osmometer head put in place. Such cells now included a great central vacuole to receive any desired cell-contents, had a plasma of an agar-gelatine-soap mixture, and a peripheral layer of lipoid which had undoubtedly penetrated the wall to some extent. The pores of the clay walls were so large that solutions of potassium and sodium would pass through them readily setting up no pressures.

That the deposit of lecithin had effectually closed the larger pores was evidenced by the fact that when cells treated with lecithin only were filled with potassium chloride or sodium chloride at 0.01*M*, the average endosmosis in 24 hours as measured by the excretion for the potassium was 6 c.c. and slightly less than 4 c.c. for the sodium. These figures represent the integration of the action of the two kations on the lipoid and their osmotic pull.

Next a series of cells with a lecithin layer and an inner layer of agar and gelatine were arranged to test the influence of saponin on

the action of potassium solutions. Two cells were used for each case and the endosmosis for 20 hours was as below:

Cell-contents.	Immersion.	Excretion or Endosmosis.
Water	Water	1.1, 1.2 c.c.
KCl 0.005*M*	Water	4 3 c.c.
KCl 0.005*M* + Saponin .005*M*	Saponin 0.005*M*	0.2, 0.3 c.c.
KCl 0.005*M* (cell treated with lecithin only)	Saponin 0.005*M*	0.0, 0.0 c.c.
KCl 0.005*M* (lecithin only)	Water	3.3, 3.5 c.c.

One of the most noticeable features of the tests with the potassium solution as above, as well as with the sodium and calcium the results of which are given below, is to the acceleration of the action of the cells so that the amount of endosmosis was greater during the first 24 hours than with cells not treated with lecithin. This is especially noticeable in cells coated internally with lecithin only.

The measurements of the action of cells filled with sodium solution are given below:

Cell-contents.	Immersion.	Endosmose.
Water	Water	1.4, 1.3 c.c.
NaCl 0.005*M*	Water	1.6, 1.5 c.c.
NaCl 0.00f*M* + Saponin 0.005*M*	Saponin 0.005*M*	0.0, 0.0 c.c.
NaCl 0.005*M* (lecithin only)	Saponin 0.005*M*	0.0, 0.0 c.c.
NaCl 0.005*M* (lecithin only)	Water	2.7, 5.6 c.c.

A series of cells with calcium as the principal salt-content showed action as follows:

Cell-contents.	Immersion.	Endosmose.
Water	Water	2.4, 0.4 c.c.
$CaCl_2$ .005*M*	Water	0.9, 0.6 c.c.
$CaCl_2$ .005*M* + Saponin .005*M*	Saponin 0.005*M*	2.6, 0.2 c.c.
$CaCl_2$ .005*M* (lecithin only)	Saponin .005*M* Exosmosis	0 0
$CaCl_2$ .005*M* (lecithin only)	Water	4.0, 0.5 c.c.

The cells containing potassium were run for 20 hours, those with sodium 16 hours, and those with calcium 24 hours. The endosmosis for two cells in terms of excretion of water is given in each case.

The control pair of cells are filled with water and set in water giving the endosmostic action of the plasmatic colloids which dissolve into the central cavity. The osmotic action of the salts in cells set in water is illustrative of relative action of the three bases in these cells. The cells containing a salt and saponin in an immersion liquid of saponin were under conditions which would liquefy the lecithin layer and make it as well as the other jellies more permeable to salts of potassium and sodium. The liquefaction of the lecithin in calcium cells apparently was accompanied by the blocking of the larger pores in the clay wall, or by the coagulative action of the calcium on the agar-gelatine jelly and the lecithin. Cells with a lining layer of lecithin only showed a sufficiently low permeability as to give marked amounts of endosmose.

The above results are of direct interest in showing that if the living cell does have a peripheral layer of lipoids the treatment with saponin might well result in its liquefaction with a resultant radical change in its permeability to salts. The observations of Boas are to the effect that some of the organic contents of the cell may exercise an influence on the action of saponin.

The principal matter of importance in this connection, however, is the possible effect of the saponin on the cell colloids beside the lipoids which would alter their permeability relations. A series of cells were given the lipoidal treatment, then lined with gelatine-agar jelly. The following results were obtained:

Contents of cell.	Immersion.	Excretion or Endosmosis.	
Water	Water	8.5 c.c.,	8.4 c.c.
Cane sugar 5 per cent	Water	8.7	8.0
Cane sugar 5 per cent	KCl 0.01*M*	4.5	6.7
Cane sugar 5 per cent. Saponin .0025*M*	KCl .0025*M* Saponin .0025*M*	1.5	1.8

The clay walls of the cells are only slowly permeable to sugar and the clay thimble with no lining layer would probably show endosmose equivalent to the amount excreted when immersed in water. When the immersion fluid contains potassium chloride the endosmose is the resultant of the opposing action of the salt and sugar. If now the sole action of the saponin were to liquefy the lecithin and render

it more permeable to the potassium, the amount of endosmose by the action of the sugar in the cell contents would have been increased. It was lessened, in fact, suggesting that the saponin exercised some influence on the condition of the wall or the plasmatic colloids. A special series to test this matter was now arranged, as noted below:

Cell-contents.	Immersion Liquid.	Excretion.	Comment.
Water.....	Water	1.4 c.c., 1.9 c.c.	Averages for 3 days
KCl 0.005*M*.....	Water	1.9 2.3	Averages for 3 days
KCl 0.005*M*.....	Saponin 0.005*M*	0.7 1.8	One cell on 2 days. Two other cells gave negative results
KCl 0.005 / Saponin 0.005*M* ...	Saponin 0.005*M*	0.0 0.0	No positive results
Sugar 5 per cent.....	Water	2.2 2.2	Averages for 2 days
Sugar 5 per cent. / Saponin 0.005*M* ..	Saponin 0.005*M*	0.0 0.0	First day
		0.4 0.4	Second day
		0.3 0.5	Third day
		0.5 1.2	Fourth day
KCl 0.005*M* / K-oleate 0.005*M* ...	K-oleate 0.005*M*	0.8 0.8	First day
		0.6 0.0	Second day

The presence of the saponin results in a diminution of endosmosis with either sugar or potassium chloride as the major constituent of the cell-contents. Such a result has only one explanation, that of increased permeability to both substances as a result of the action of the saponin on the clay wall and the plasmatic layer.

After the above readings had been made the cell which contained potassium and was immersed in water was shifted to an immersion in saponin 0.005*M*. Exosmosis resulted immediately, which would be negative osmose, as the osmotic action of the salt solution would be much greater than that of the dilute saponin without. The cells containing water only were now filled with KCl 0.05 and immersed in saponin 0.005*M*. One gave 0.5 c.c., then showed exosmosis. The other gave 2 c.c. and 1.8 c.c. on successive days.

An untreated clay thimble was filled with the combined sugar and saponin and set in saponin, with the result that an endosmosis of 1 c.c. was measured in the first day. Another untreated thimble filled with sugar in 5 per cent. solution gave 0.5 c.c. endosmose in 1 day, which was below expectancy for these preparations. The cell

with an agar-gelatine plasma filled with a 5 per cent. cane sugar solution gave averages of over 2 c.c. daily when immersed in water, but action ceased when it was transferred to a 0.005*M* saponin solution.

It is evident that the presence of saponin with sugar or salt solutions in the contents of the clay thimble or the cell, or in the immersion fluid, lessens endosmose, presumably by increasing the permeability of the membranes, or layers of jelly and the firmer wall.

Some of the agar (3 parts) -gelatine (2 parts) jelly used in making the plasmatic layer of the cells was dehydrated, coming down to a plate 0.12 mm. in thickness. Trios of section were hydrated under the auxograph with the following increases in thickness at 14–16° C.:

Water	2200 per cent.
Saponin 0.01*M*	2830 per cent.
KCl 0.01*M*	2250 per cent.
KCl + Saponin 0.01*M*	1875 per cent.

The actual hydration in the saponin was less than in water, that in KCl was still less, while the combination of the salt and the saponin restrict hydration still more. The action of the saponin on a plasma of the above type would therefore be to lessen permeability alone and in the presence of the salt. This would tend to increase osmotic action, if the plasma alone were concerned. It must be concluded, therefore, that the saponin has no action in the artificial cell except that which would lessen permeability of the plasma and increase that of a lipoidal layer and of the porous outer wall.

Attention was now turned to the more difficult task of interpreting the action of living and dried cell-masses in solutions which might theoretically affect the peripheral layer. The measurements of Kahho were taken from roots fully hydrated in distilled water. When such roots were placed in KCl at 0.22*M* an initial shrinkage of 11 per cent. ensued within a few minutes, to be followed by an expansion which regained 4 per cent. of the shrunken length.

The flat joints of *Opuntia* were chosen as the material for my own tests, as the composition and general behavior of these plants

has been a subject of study at the Desert Laboratory for many years. Sections about 1 cm. square and having the thickness of the joint 10.5 mm. were placed in various solutions at 14–18° C. and their changes in thickness recorded by the auxograph as below:

Solution.	Increase.	Comment.
Water	31 per cent.	In 20 hours followed by shrinkage of 2 per cent. in following 30 hours
KCl 0.01*M*	31 per cent.	Continuous swelling for 50 hours
KCl 0.0075*M* + Saponin 0.005*M*	15 per cent.	In 12 hours followed by shrinkage of 8 per cent. in 40 hours
Saponin .01*M*	11 per cent.	In 6 hours with shrinkage of 7 per cent. in 44 hours

Shrinkage in the two cases treated with saponin would have reduced the sections to original dimensions in a few hours more.

It is to be noted that Boas used 2.5 per cent. solutions of saponin in the treatment of the higher plants, which would be equivalent to about a 0.003*M* solution.

A test was made with slices cut longitudinally from the median portion of the joint consisting chiefly of large, thin-walled parenchyma. The average thickness of the trios in the swelling dishes ranged from 3.5 to 4 mm. and the increase was calculated on the original measurements.

The following increases were obtained:

Solutions.	Increase.	Comment.
Water	40 per cent.	Very slight shrinkage after 30 hours
KCl 0.005*M*	38 per cent.	Very slight shrinkage after 30 hours
KCl 0.005*M* + Saponin .005*M*	8 per cent.	Decided shrinkage after 6 hours which carried the pen back near the base line in 40 hours
Cane sugar	1 per cent.	
Saponin 0.01*M*	8 per cent.	
Saponin .005*M*	9 per cent.	Decided shrinkage after 6 hours which carried the thickness back nearly to the original after 40 hours

At the conclusion of the test as above the ones hydrated in water and in KCl were put in a saponin solution at 0.005*M* with a shrinkage, most rapid in the sections first swelled in KCl, which reduced them nearly to their original dimensions.

Another series of slices of *Opuntia* about 2.5 mm. in thickness were first allowed to hydrate in water for 6 hours, in which time an increase of 35 to 40 per cent. was noted. The water in the dishes was now pipetted off and replaced with other solutions. Saponin 0.005*M* and a mixture of saponin 0.005 and KCl 0.005*M* resulted in a rapid shrinkage to about the original dimensions in 10 hours. KCl 0.005*M* had no effect on a trio of sections which quickly shrunk when the salt was replaced with saponin 0.005*M*.

The maximum swelling of thin sections of dried material of *Opuntia* was reached at a concentration of saponin between 0.001*M* and 0.005*M*. Samples which swelled 150 per cent. in water made an increase of 188 to 200 per cent. in such saponin solutions. Other samples which swelled 220 per cent. in water showed increases of 260 to 320 per cent. in saponin at the above concentrations. The increase was practically identical with that of water at 0.0002*M* and at 0.01*M* and stronger. The sections first dried and then hydrated as above showed no shrinkage at the end of 36 hours, even in the stronger solutions. The action seems to be one purely of imbibition of water by walls, mucilages, and lipoids. It is not affected by KCl.

The hydration of living sections in saponin shows the effect of altered permeability. A series of thin slices of living tissue were hydrated in a graded series as below, and after full expansion had been reached a neutral salt, NaCl, was added to increase permeability in some sections. The results are given below:

Solution.	Swelling.	Time.	Comment.
			0.005*M*
Water.....	33 per cent.	8 hrs.	Replaced by NaCl 0.01*M* and shrinkage set in steadily
Saponin 0.005*M*.....	13	15 min.	Followed by rapid shrinkage
Saponin 0.001*M*.....	27	4 hrs.	Followed by more gradual shrinkage
Saponin 0.0002*M*....	30	4 hrs.	Replaced by NaCl 0.005*M* with shrinkage at rate identical with that in water
Saponin 0.000,04*M*...	39	6 hrs.	Replaced by NaCl 0.005*M* with gradual shrinkage at same rate as in above and in water

The initial swelling of the sections is met and canceled most quickly in the stronger solutions, which increase the permeability of

the cell and allow the escape of its contents. Whether wall and plasma are equally changed is not shown. It is important to note that such increased permeability results in the stronger solutions without the addition of a salt as in Boas's experiments.

Increase of the hydrogen-ion concentration did not modify the action of the saponin in the single series of tests carried out as shown below in which thin slices of living tissue were hydrated.

Solution.	Swelling.	Time.
Water	20 per cent.	2 hrs.
Saponin 0.005*M* HCl 0.005*M*	9	20
Saponin 0.005*M* KCl 0.005*M*	9	10

The presence of the salt, however, appeared to speed up the process of imbibition and to hasten the shrinkage.

Sections of living tissue such as the above show a swelling slightly less than that in water when hydrated in acid at PH 2, due presumably to increased permeability. KHO at 0.01*M* also gives a swelling slightly less than water, at PH 12. Such a result would be much more complicated, as the hydroxide may affect lipoids as well as the proteins of the plasma. Sections which increase about 75 per cent. in water swell only 31 per cent. in KHO 0.2*M*, 67 per cent. at 0.05*M*, and 70 per cent. at 0.01*M*, and the maximum lies near this concentration, a lessening swelling appearing in concentrations of 0.005*M*.

Dried sections hydrated in a series of concentrations of HCl which swelled 240 per cent. in water did not reach this figure in HCl 0.01*N*. At weaker concentrations no graded series of values was obtained, but increases of 255 to 300 per cent. were measured. These reactions would be similar to hydration values of agar-gelatine biocolloids in which the pentosanic and the albuminous components were nearly equal in quantity. Similar sections which swelled 185 per cent. in water increased 200 per cent. in KHO at 0.01*N*, 250 per cent. in a 0.005*N* solution, 210 per cent. in a 0.001*N* solution, and gave the same value as water in KHO at 0.0002*N*. These hydration reactions are also in consonance with the relative swellings in water

and hydroxide of mixtures of agar and gelatine nearly evenly balanced.[13] Although the lipoids present would be liquefied in the stronger solutions, the effect of their presence is not discernible in the action of the dried sections in which the permeability of the wall and other layers has reached the maximum.

The numerous corrections and amendments to the plasmolytic method of estimating permeability and tonicity of cell-contents are suggestive of the complexity of the factors which enter into the exchange between the cell and the medium. An average of sixty per cent. of the osmotic pressure of the cell sap is due to electrolytes. These with the non-electrolytes affect or determine the degree of hydration of the constituents of the plasma and the wall, upon which permeability depends directly. Such action is with but little reference to the isotonic values of the substances concerned. A knowledge of the principal features of the hydration reactions of the plasmatic constituents, and of the cell-wall under the influence of cell-contents and medium is therefore fundamental to any comprehension of the passage of material through the membranes of the plant.

According to the recent work of Bartell and Sims, swelling or increase by hydration may be the result of the action of several forces. Whether their conclusion that a solution tending to exercise negative osmose increases hydration of the membrane, while conditions which shrink the membrane act positively is in agreement in all of its implications with those of Kahho as to penetration and hydration, is not yet clear.[14] Some of the newly disclosed possibilities of the intervention of the lipoids are to be taken into account in any consideration of the passage of material into or out of the cell with resultant changes in volume which constitute the essential features of growth.

[13] MacDougal and Spoehr, "The Components and Colloidal Behavior of Plant Protoplasm," *Proc. Amer. Phil. Soc.*, 39, 150–170, 1920. See pages 156, 157.

[14] Bartell, F. E., and Sims, L. B., "The Relation of Anomalous Osmose to the Swelling of Colloidal Material," *Jour. Am. Chem. Soc.*, 44, 289–299, 1922.

THE DESERT LABORATORY,
CARNEGIE INSTITUTION,
TUCSON, ARIZONA, April, 1922.