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The distentive agencies in the growth of the cell.

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The newly separated vegetative cell including that of the simpler animals such as the Protozoans is a minute mass of protoplasm in which there are at first no lacunæ or breaks of any size, and the externally bounding layer is of extreme thinness and tenuity. The enlargement of expansion of the protoplast in this stage is chiefly one of formation or accretion of additional colloidal material and its hydration to a point where the water content is 50 to 500 times that of the dry weight of the included material.

My experiments of the last five years show that substances known to accelerate or facilitate growth also carry the hydration of living and dead cell masses and of pentosan-protein colloidal masses to a point beyond that which may occur in pure water. Such amino compounds, acidic and basic, as asparagin, alanin, glycocoll, phenyl-alanin and histidin, hydroxides and chlorides of potassium, sodium, magnesium and calcium in concentrations from 0.001 M. to 0.000,01 M. hydrochloric acid in the same range and water-soluble B yeast-vitamine Harris are included in the list and these are concentrations of biological occurrence. The enlargement of the cell in this stage must be due directly to imbibition and swelling as the vacuoles are not yet formed,

although the fundamental unity of swelling and osmosis is to be recognized. In a second phase of growth, characteristic of plants and well illustrated by yeasts and bacteria, synergetic cavities appear in the plasmatic mass, and these known to the morphologist as vacuoles enlarge presumably by osmotic action, to such extent that their volume may comprise three fourths of the space of the cell.

Substances of four main groups, carbohydrates, proteins, soaps and lipoids which may be in the colloidal state of a reversible gel are entangled or intermixed in the protoplasmic mass. The components which tend to lower surface tension most would be carried to the surface, and it is to such implied causes that we must look in determining the character and derivation of the so-called plasmatic membrane, the permeability of which is of the greatest importance in the physical action of the cell, and of the wall which encloses the whole. Much controversy has raged as to nature and composition of this membrane. Its composition and character must inevitably depend upon the character of the colloidal mass which it bounds and from which it was formed. Morphologically inseparable, it yields no molecular features under the ultra-microscope, which would distinguish it from the mass. That it is a polarizable protein layer, or pentosan anhydride or that it is an albumen-lipoid mosaic are all theses which fall to the ground when tested by the known laws of colloidal action, or by the phenomena of cell-behavior.

Furthermore the conclusions of Hansteen-Cranner¹ that the ground substance of living matter is a colloidal meshwork of lipoidal matter which runs through walls from cell to cell and which takes the form of a highly irregular deposit between wall and plasma, may be taken as a highly particularized interpretation of facts he has uncovered as to the occurrence of these lipoids. All jellies present will be entangled in the protoplasmic mass, and what may be or what may not be protoplasm is a question the answer to which lies not in the realm of morphology but of energetics. It may well be that it is to the action of the lipoidal substances and soaps that we may look hopefully for the solution of some of the anomalies in permeability.

¹ *Ber. d. Deut. bot. Gesell.*, 1919, xxxvii, 380.

The hydration reactions of living cell masses, of dried tissues and of plates of colloids simulating the composition of living matter having been determined, attention was next turned to experiments which might afford a means of estimation of the action of such material in the vacuolated cell. In brief it was found necessary to devise a new or improved form of a little used type of artificial cell. It was seen that such a device should be one in which the character of the wall might be changed, the plasma represented by various jellies with consequent variations in the contact zones (phase boundaries), and furthermore that such a cell could be subjected to a range of solutions, externally and internally comparable to those of the living cell. Such a cell consists essentially of a capsule of a gel enclosed in walls of varying porosity and other mechanical characters. To facilitate determinations of action the capsule must be fitted with an osmometer head for measurement of volume or pressure. The experiments which have been made to this date have been with cells in which agar, gelatine and various mixtures of these with potassium oleate, calcium oleate, lecithin and other substances have been used as the "plasmatic" layer and the outer wall was represented by platinum gauze, Whatman's double thickness filter- or extraction-thimbles, wooden cups, and by clay cups such as are used in the Livingston atmometer. The ranges of conditions offered for the study of absorption and of osmose are fairly equivalent to those of the living cell.

If we now take such a cell and set it in operation to ascertain what may happen when the vacuoles begin to appear by syneresis in the protoplasm, using for example one in which the jelly forms a layer about 3 mm. in thickness on the inner side of the wall with a cell content of 30 to 40 c.c. we may get what may be taken to be the initial action in such a case by filling it with water and setting it in water. The first thing to occur in such a case will be the hydration of the jelly to a point approaching its maximum. Coincidentally the solution or dispersion of material from the inner surface of the layer begins in a manner which should be practically identical with that which ensues with the enlargement of the vacuoles. The action of these free particles of colloidal material results in a mounting osmose which measured by the exudation

from the outlet of the cell reaches its maximum between 75 and 100 hours at 15° C., at about 3.5 to 4 c.c. daily and, when measured in terms of pressure set up by a cell lined with agar the magnitudes are in a range not far from those given for gum acacia. This secretion or exudation was found to continue for as long as 70 or 80 days in cells with a plasmatic lining of a mixture of agar, gelatine and potassium oleate, during which time the total excretion would amount to two and a half times that of the capacity of the cell, that is about 75 or 80 c.c. The dispersion and osmotic action of the plasmatic colloids in the vacuoles as illustrated must be recognized as one of the initial factors in the distention and turgidity of the cell. Diffusion of sugars and their decomposition products, and of amino-compounds of various kinds into the vacuole, must also ensue from a constantly renewed supply furnished by the metabolic activity of the protoplasm. Salts included in the plasma must also be diffused into the vacuolar liquid, and it is to electrolytes that Pfeffer and others attribute as much as sixty per cent. of the total osmotic capacity of the cell, which in the higher plants shows a potential from 3 to as high as 150 atmospheres as determined by J. A. Harris and others using cryoscopic and plasmolytic methods. When it is realized that the partial pressures which must be attributed to the electrolytes are much higher than those which are ordinarily found in soil solutions or in media in which simple organisms live the manner in which the higher concentrations are accumulated in the cell becomes as much of a problem as that offered by glandular action in the body of the animal.

Some measurements of cumulative action in building up turgidity have been made, in which it was shown that a cell mass which might be plasmolyzed at a certain high concentration if first immersed in a series at lower concentrations might be led up to endurance of this concentration without loss of turgidity. A similar action may be demonstrated by the artificial cell described above. When lined with an agar-gelatine-potassium oleate mixture it shows a tonicity of about 0.00 M. potassium chloride, showing no positive action when filled with water and immersed in a concentration above this. If however it is first allowed to get into full action in the above concentration it may then by small

steps be moved up to where it shows a tonicity equivalent to 0.005 M. This is a short series of small steps, but it is one shown by a cell in a static condition in the sense that none of its parts are being renewed or altered by metabolism, but most important of all it is in the direction of cumulative turgidity. Such action may or may not however imply any accumulation of electrolytes but it is proven that the cell contents by changes in material partly or entirely derived from the jelly or plasma shows an increasing osmotic pressure measured against potassium chloride. The actual diffusion of the salt into the vacuole has not been measured.

It is the external zone or phase boundary of the plasmatic layer to which attention has been chiefly directed in studies of permeability and diffusion of salts into and out of the cell. Its original structure at the moment of formation of the new cell is in all probability that which might be predicated for the external layer of any such a semi-liquid mass of jelly, composed of albumens, pentosans, soaps and lipoids, the two last-named forming but a small proportion of the dry weight of the mass, but by their surface tension relations would tend to assume a peripheral position. Following this initial arrangement it is also to be seen that compounds of these groups would continue to be added from the products of a continuing metabolism. The resulting formation would present a picture widely different from that of the parchment membrane to which it has been so often compared. In accordance with this view the shrinkage of the protoplasm from the wall in plasmolysis is not accompanied by a detachment of a membrane from the wall but by an actual rupture or partial destruction of the peripheral layer. When in addition my own results to the effect that the hydration or swelling of dried sections of biocolloids is accelerated by balanced solutions of sodium, while the osmotic action of artificial cells lined with the same biocolloids is seen to be not affected by antagonisms, a revision of some widely prevalent views as to the nature of permeability seems to be required.

If we now turn attention to the outer wall of the vegetable cell we see it developing from a semi-liquid cell-plate to a condition approaching that of densely layered and infiltrated structure;

from a condition of extreme permeability to one in which solutions of common salts may not pass through it. It is of course to be recalled that not only does the wall change with its growth but that it may do so quite irregularly so that the wall may have impermeable areas and others of ready permeability.

Some possibilities of the influence of the wall on the exchange between the protoplasm and its environment are suggested by effects in negative osmose which have been obtained by the use of the artificial cell, this term *negative osmose* being used to denote increase in volume of the solvent in the cell against a solution of higher concentration external to the cell. Thus when an osmometer comprising only the outer clay or wooden wall of the artificial cell is filled with water and immersed in a solution of calcium chloride at various concentrations ranging from 0.0035 M. upward negative action ensues, the contents increase in volume and exudation takes place. If the cell be given its artificial plasmatic lining the effect continues, overbalancing the positive osmotic action of the colloidal components dissolved in the cell. If the electrolyte be placed in the cell its action dominates and exosmosis, being "negative" osmose, ensues. These results so far as they have been examined being consonant with those of Bartell, who has recently made many measurements of negative osmose through porcelain plates.

A summarization of the facts and conclusions cited in this paper may be made as follows:

1. All substances which may appear in the cell in the colloidal condition of reversible gels must be taken into account in any adequate interpretation of cell-action, particularly growth. Albumin, pentosans, soaps and lipins are thus to be taken into consideration in cell-mechanics. The findings of Hansteen-Cranner to the effect that lipins accumulate in quantity at the periphery of the protoplast and run in a meshwork from cell to cell and through the cell does not justify his conclusion that such material is the ground substance of living matter. The actual identity of living matter is not a question of structure but is one of integrated action, not one of equilibria but one of energetics.

2. All attempts to find separable membranes to protoplasts by dissection, and by microscopic and ultramicroscopic methods have

ended negatively. The so-called membranes are peripheral layers resulting from the action of surface tension in masses of widely varying composition under a wide range of external conditions.

3. All of the substances known to promote growth which have been tested have been found to accelerate hydration in living cells, dead cell-masses and protein-pentosan,—soap jellies at particularized concentrations from 0.01 M. to 0.000,01 M. and through a range of P_H from 4 to 11. Such action would represent the action of young cells. The second phase of growth of the cell is accompanied by the synergetic formation of vacuoles setting up conditions, which have hitherto been interpreted in terms of osmotic equilibria.

4. The demonstration of the fact that polarizable separable membranes are not formed, that the peripheral layer is not dominantly proteinaceous, and that osmotic potentials are accumulated in the cell far overbalancing that of the medium makes evident the necessity for some new interpretations.

5. The attempt is recorded to make up and operate an artificial cell the outer wall of which should be a fixed colloid of clay, wood, or parchment, and a lining or plasmatic layer of reversible gel, simulating protoplasm, with a view to obtaining information as to the accumulation of electrolytes in the cell with resultant overbalancing osmotic potentials.

6. Such cells filled with water and immersed in water show an intake and excretion for a continuous period of 60 to 80 days at 15° C. during which time the vacuolar content of the cell is replaced two or three times.

7. The artificial cell may be arranged to show exosmose in its earlier stage, followed by endosmose, and negative osmose by the action of calcium or magnesium salts as vacuolar contents or as immersion media. Cells which have a tonicity equivalent to 0.003 M. potassium chloride when filled with water may be raised to a tonicity of 0.005 M. by immersion in a series of increasing concentrations, after a manner which has been used in raising the tonicity of living cell-masses.

8. The course of metabolism, the action of light and the transpiration of water may be held to account for the concentration of carbohydrates and electrolytes and the conversion of sugars

and proteins to more highly osmotically active forms in leaves and other organs. There occurs however in many tracts of the plant an accumulation of material, in which diffusion or excretion may be said to work against osmosis after the general manner illustrated by glandular action in animals. The use of the artificial cell promises results of interest in the solution of such problems.

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The bacterial content of the stomach as influenced by saliva.

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The fractional method of gastric analysis makes possible a bacteriological study of the stomach which includes not only the active cycle of digestion, but the resting phase as well. So far as could be ascertained, no quantitative bacterial studies employing this method have heretofore been reported. In fact, very little data concerning the types of bacteria in the stomach at different stages of digestion have appeared in the literature beyond the work of Cotton.¹

In a previous paper,² the writer has shown that repeated analyses on the same individual within a short period of time—while the physical and mental condition remain practically unchanged—yield different curves. These curves from the same individual often vary as much from one another as the difference between the curves of different individuals. This holds true likewise for the average fasting contents. Therefore the conclusion was reached that single determinations by the Rehfuß method are not sufficient upon which to base valid conclusions since they do not take into consideration individual variations. In bacteriologic studies of fractional gastric analyses carried out repeatedly on psychotic patients and normal individuals, in only one half the instances was there any correlation between high acidity and low bacterial numbers or vice-versa. Upon close observation it be-

¹ Cotton, H. A., *N. Y. Med. Journ.*, 1920, cxi, 672-677; 721-727; 770-775.

² Kopeloff, Nicholas, read before the Brooklyn Neurological Society, October 19, 1921 (in press).