The Properties of Colloidal Systems. IV.—*Reversible Gelation in Living Protoplasm.*

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Protoplasm itself, using the name to express living substance in its simplest, undifferentiated form, is generally recognised as having the properties of a somewhat viscous liquid. This fact was realised as long ago as 1864 by Kühne (1864). As usually seen under the microscope, it contains suspended in it a number of granules of a great variety of dimensions and properties. But in the pseudopodia of an amœba or of a leucocyte, when examined by the ordinary method of illumination with transmitted light, it appears completely devoid of contents or structure. As Hardy (1899) showed, the various networks and similar arrangements seen in fixed preparations are produced by the action of the reagents used, although they indicate that protoplasm contains matter in the colloidal state.

The use of the method of brilliant lateral illumination on a dark ground (so-called "ultra-microscope") has led to the detection of particles in protoplasm which are too minute to be visible by ordinary illumination. Of course, these particles, being comparable in dimensions with the mean wave-length of light, are not seen in their true dimensions or form, but by their diffraction discs. Remembering that the late Lord Rayleigh showed that the more intense the illumination, the more minute are the particles that it is possible to detect, I tested the result of increasing the intensity of the dark-ground illumination applied to the apparently clear and structureless pseudopodia of large amœbæ. The broad flat pseudopodia of a species, which appeared to correspond to *Amœba princeps* (Leidy), were found to be most appropriate for the purpose. A paraboloid condenser, made by Zeiss, was used in most cases. The source of light was the positive crater of a small arc lamp with carbons at right angles to one another. The rays were made parallel by a condenser, and passed through a cell with parallel sides, about 5 cm. apart, before falling on the mirror of the microscope. The water-cell was found to be necessary on account of the heat otherwise transmitted being sufficient to kill the organisms. In order to obviate the injurious effect of any ultra-violet rays which might be transmitted through the system, quinine sulphate was added to the water. The objective used for the majority of the observations was an excellent $\frac{1}{6}$-inch dry lens made

by Swift, which was found to admit of magnification by fairly high-power oculars, such as No. 12 compensating of Zeiss. Other methods, such as that of an objective as sub-stage condenser, with a central stop in the observing objective to cut out direct rays, were tried; but the paraboloid was found to be the best on the whole.

In practice, the observations themselves are somewhat trying, on account of the extreme brilliancy of the larger particles when they come into the field of view. But under favourable conditions, and with sufficient intensity of illumination, it is not difficult to see that the clear protoplasm of the pseudopodia contains an immense number of very minute particles, shown by their bright diffraction images. They are in vigorous Brownian movement, and it is scarcely possible to distinguish separate particles, on account of their number. The general appearance is that of a shimmering tremulous movement in the field of view.

This Brownian movement is one of the most convincing proofs of the liquid nature of the system. A simple experiment shows the fact. If a cake of water-colour gamboge be rubbed in a drop of 5 per cent. gelatine solution on a warmed microscope slide, covered and immediately examined under the microscope, the particles exhibit Brownian movement in the usual manner. As the slide cools and the solution sets into a jelly, the movement becomes inert and finally ceases, the particles becoming fixed in the meshes of the network. On warming, the movement reappears. The fact was made use of by J. Duclaux (1908) for the purpose of enumerating the particles in colloidal ferric hydroxide.

It seems, from Kühne's description of his valuable observations, that he was unable to satisfy himself that the particles which were visible to him possessed Brownian movement, or, as he called it, "molecular movement," as distinct from the translational movements due to currents in the protoplasm. If he had been able to use dark-ground illumination, there is no doubt that he would have detected them, since they can be distinguished by the vibratory motion of the particles even when they are being carried along. They have, indeed, been described by Gaidukov (1910) and by Price (1914) in plant cells, by Chambers (1917) in various animal cells, by Mott (1912) and by Marinesco (1912) in nerve cells.

Protoplasm belongs, then, to the class of colloidal solutions called by Graham (1864) "hydrosols." Now, as Graham showed, many of these under certain conditions change their state, becoming solid, in the sense that they become fixed in shape. This phenomenon is familiar in the "setting" of gelatin when cooled, and the new systems are called "hydrogels." The series of changes taking place was investigated by Hardy (1900). It is

sufficient for our present purpose to call attention to the fact shown by the experiment with gamboge given above, namely, that liquid properties cease and a fixed structure makes its appearance.

Certain facts noticed by Kühne, in his experiments on electrical stimulation of protoplasm, suggest that a reversible change of the kind referred to may occur in this colloidal system, although Kühne did not interpret them as of such a nature. It is to be remembered that his work was done very soon after the publication in 1861 of Graham's investigations on colloids which had not then become a part of common physiological knowledge. An observation made by Gaidukov (1910, p. 58), in the course of his work on the phenomena shown in living protoplasm by dark-ground illumination, suggests that "spontaneous" changes from sol to gel take place. This observer noted that the movements of the protoplasm in the cell of *Vallisneria* appeared occasionally to become arrested at a spot, while the Brownian movement of the particles in it ceased. Presently, the Brownian movement became visible again, and immediately afterwards the general protoplasmic circulation was resumed. According to Chambers (1917) there is a periodic reversal of the sol to the gel state, and *vice versâ*, in the process of cell-division, the greater part of the cell being in the state of gel when the aster is fully formed. In fact, the appearance of the aster is associated with gelation of the protoplasm. Leblond (1919) describes the phenomenon as occurring in conjugation, as well as in cell-division. In fact, the occurrence of reversible gelation seems to be a regular condition of cell activities. Some observers have described the appearance of a reticular structure. But, on account of the ease with which a series of points gives rise by diffraction to network images, care is needed in the interpretation of such images. Nevertheless, Hardy (1900) has described the formation of networks in moderately strong solutions of gelatin in dilute alcohol, so that there is reason to admit the possibility of their production in protoplasm.

The experiments which I made were for the purpose of seeing whether this reversible state of gelation could be induced at will by means of electrical stimulation. The method used for stimulation was the same as that of Kühne. Two thin blunt-pointed pieces of platinum foil were cemented on a microscope slide, so that there was a gap of about 2 mm. between the points. A drop of water containing amœbæ or other cells was placed between the points and a cover-glass dropped on the top. The platinum strips can readily be connected to the terminals of the secondary coil of an induction apparatus by resting the bared ends of fine copper wire on them and placing small weights on these. It was found to be a matter of some difficulty to adjust the strength of the stimulus to an appropriate

value. If too strong, the organism "explodes," driving out its contents into the water, where they rapidly become dispersed. But even if the current is not strong enough to produce any immediate effect, it is necessary to cut it off the moment that any trace of contraction or other effect shows itself. A very weak current, if left on for a sufficiently long time, kills with disintegration. Moreover, the protoplasm may be "killed," in the sense of permanent cessation of movement, although no immediate breaking up may take place. Since, therefore, if recovery is to be expected, it is not permissible to continue the stimulus for more than a brief time, the observations must be made rapidly.

It is a comparatively simple matter to convince oneself of the correctness of Kühne's statement with regard to the cessation of the flowing movements of the larger granules into and out of the pseudopodia. This is, no doubt, rightly stated to be due to the "contraction" of the organism into a more or less spherical shape, with simultaneous arrest of the protrusion and retraction of the pseudopodia. But it is clear that this observation does not necessarily imply a change of the protoplasm into the gel state. This latter can only be tested by examination of the Brownian movements of the minute particles visible by dark-ground illumination. The most satisfactory place to make this observation is the clear protoplasm of the outer part of the pseudopodia, which is free from the to-and-fro movement of the large granules of the central protoplasmic mass. It is generally possible to make use of a stimulus not strong enough to cause sudden retraction of the pseudopodium as a whole. In a successful experiment, the effect is very striking. The continuous shimmering tremulous movement of the bright points, due to their Brownian movement, ceases almost instantaneously, as if the liquid protoplasm had been frozen. As soon as this happens, the stimulation is stopped, and, apparently, almost at the same time, the Brownian movement and the flowing pseudopodial extrusion recommence.

It is significant that the Brownian movement does not cease during natural pseudopodial movement of the protoplasm. This fact might, perhaps, be regarded, especially by those who look upon the gel state as an accompaniment of cell activity in general, as evidence that the protrusion of pseudopodia is the result of differences of surface tension at the contact between protoplasm, water, and solid surface on which the organism rests. But the mode of production of pseudopodia is as yet a matter of dispute.

If the electrical shock in the experiments described above has been too strong, so that the organism is killed, but not so strong that "explosion" takes place, the fixed state of gelation is permanent; the sol state does not return until disintegration sets in. This is in agreement with the

statement of Gaidukov that death is associated with an irreversible coagulation. It appears, however, from some observations of Kühne, that the lethal gel state begins at a later stage to show Brownian movement again. Kühne interpreted this reappearance as being due to absorption of water. It may indicate the commencement of autolysis. Sherrington (1894, p. 188), in his observations on leucocytes, regarded the appearance of Brownian movement as a sign of approaching death. It seems probable that the second stage may come on more quickly in these cells than in *Amœba*. Leucocytes may consist of more viscous protoplasm and the granules observed by Sherrington may have been too large to show Brownian movement until the post-lethal changes had reduced the viscosity of the medium. I have not made any observations on these cells. Pus cells, according to Sherrington, show Brownian movement of the particles which they contain.

Although many plant cells are very favourable objects for the investigation of the movements of protoplasm, I found them less so for the brilliant illumination required for the observations of the present paper. The cell wall, by its dazzling brightness, renders the detection of changes in the protoplasm in contact with it a matter of difficulty. In *Nitella*, probably owing to the relative delicacy of the cell wall, I have, however, been able to observe phenomena similar to those in *Amœba*. In *Spirogyra*, the central vacuole contains numerous granules in Brownian movement, and I was unable to satisfy myself that their movement could be distinguished from that of the particles in the protoplasmic layer itself. In the staminal hairs of *Tradescantia*, although one could recognise a cessation of Brownian movement in the protoplasmic trabeculæ on stimulation, it would have scarcely been possible to feel assured of it without previous acquaintance with its appearance in *Amœba*, for the reason referred to in the case of *Spirogyra*, namely, the presence of particles in the cell sap, on which stimulation has no effect.

Summary.

With intense dark-ground illumination it is possible to see that the apparently clear pseudopodia of *Amœba* are filled with numerous very minute particles in Brownian movement; thus affording further evidence of the liquid, hydrosol, nature of simple protoplasm.

By electrical stimulation, this sol can be reversibly changed into the gel state, evidenced by the sudden cessation of the Brownian movement.

REFERENCES.

Chambers, Rob., Jr., "The Cell Aster: a reversible gelation phenomenon," 'Journ. Exp. Zool.,' vol. 23, p. 483 (1917).

Duclaux, Jacques, 'Comptes rendus,' vol. 147, p. 131 (1908).

Gaidukov, N., 'Dunkelfeldbeleuchtung und Ultramicroscopie in der Biologie und in der Medizin' (1902), Jena, Fischer, 84 pages.
Graham, Thos., "Liquid Diffusion applied to Analysis," 'Phil. Trans.,' vol. 151, pp. 183–224 (1861).
Graham, Thos., "On the Properties of Silicic Acid and other Analogous Colloidal Substances," 'Trans. Chem. Soc.,' vol. 2, pp. 318, 327 (1864).
Hardy, W. B., "Structure of Cell Protoplasm," 'Journ. Physiol.,' vol. 24, pp. 158–210 (1899).
Hardy, W. B., "The Mechanism of Gelation in Reversible Colloidal Systems," 'Journ. Phys. Chem.,' vol. 4, pp. 254–273 (1900).
Kühne, W., 'Über das Protoplasma,' Leipzig, Engelmann, 158 pages (1864).
Leblond, É., "Le passage de l'état de gel à l'état de sol dans le protoplasma vivant," 'C. R. Soc. de Biol.,' vol. 82, p. 1150 (1919).
Leblond, É., "L'état de sol dans ses rapports avec l'activité fonctionnelle du protoplasma," 'C. R. Soc. de Biol.,' vol. 82, p. 1220 (1919).
Marinesco, G., "Forsch. über d. kolloid. Bau d. Nervenzellen und ihre erfahrungsgemäss Veränderungen," 'Kolloid-Zs.,' vol. 11, pp. 209–225 (1912).
Mott, F. W., "The Bio-Physics and Bio-Chemistry of the Neurone," 'Brit. Med. Journ.,' September 28, pp. 780–784 (1912).
Price, S. Reginald, "Structure of the Plant Cell by Dark Ground Illumination," 'Ann. of Botany,' vol. 28, pp. 601–632 (1914).
Sherrington, C. S., 'Roy. Soc. Proc.,' vol. 55, p. 161 (1894).

Studies of Photo-synthesis in Fresh-water Algæ.—1. *The Fixation of both Carbon and Nitrogen from the Atmosphere to form Organic Tissue by the Green Plant Cell.* 2. *Nutrition and Growth produced by High Gaseous Dilutions of Simple Organic Compounds, such as Formaldehyde and Methylic Alcohol.* 3. *Nutrition and Growth by means of High Dilutions of Carbon Dioxide and Oxides of Nitrogen without Access to Atmosphere.*

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The two most primæval and most fundamental chemical processes for living organisms are those two in which their living substance is synthesised from inorganic sources with uptake of energy. By one of these carbon is built into organic compounds, starting with the oxidised carbon dioxide of the