ON THE STRUCTURE OF CELL PROTOPLASM.

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Part I. The Structure produced in a Cell by Fixative and Post-mortem change. The Structure of Colloidal matter and the Mechanism of Setting and of Coagulation.

CONTENTS.

SINCE Brücke made the deduction that the complex phenomena of the life of the cell can only be exhibited by structured matter nearly forty years have elapsed. During that period a great part of the facts and theories of modern physiology have been discovered and formulated; and during that time more than fifty workers have endeavoured to convert Brücke's deduction into an induction founded upon the direct investigation of the structure of the cell-substance. It is not going beyond the facts to say that in spite of their labours not one single point has been placed beyond the domain of ardent controversy; almost nothing has been added to the settled body of knowledge.

At the present moment the living cell-protoplasm[1] is regarded by many as being composed of two substances, one of which is disposed as a contractile net according to some, as a relatively rigid framework according to others, or as free filaments. Other workers again regard cell-protoplasm as being built up of a more solid material, and of a more fluid material which occupies the minute spaces or vacuoles which are hollowed out in the former. Still others view it as a homogeneous jelly holding granules. Lastly there are still those who deny the truth to all these views and maintain that the living cell-protoplasm is homogeneous in so far that it does not manifest the relatively coarse structure which these theories ascribe to it. Its peculiar and transcendent qualities are according to them associated with molecular and not molar structure.

In this paper I endeavour to show that, if one puts on one side the confusion which has arisen from the inheritance of two nomenclatures of the parts of the cell, the one due to the botanist Hanstein, and the other started by the zoologist Kupffer, this lack of consonance in the views held as to the structure of the cell-protoplasm is traceable in the main to the fact that they are largely based on details of structure visible both in fresh and in fixed cells which are the result of the physical changes which the living substance undergoes in the act of dying, or at the hand of fixatives.

Flemming[2] was perhaps the first to draw special attention to the fact that the appearance of structure in the cell-protoplasm might be due to the action of fixatives or to post-mortem changes. He showed

[1] Throughout this paper the words "cell-substance" are used to indicate the whole of the cell exclusive of the nucleus; and the words "cell-protoplasm" to indicate the cell-substance with the exception of secretory granules, glycogen or fatty masses, or other stored (paraplastic) products.

[2] *Zellsubstanz, Kern, und Zelltheilung.* Leipzig, 1882, p. 51.

that a fine net structure is produced when the sap of a vegetable cell is fixed with osmic acid,—this net therefore is an artifact due to the action of the fixing reagent.

The possible origin of structure in the dead cell-substance which is not present in the living state, and which therefore is artifact for the purpose of the present discussion, appears to me to be threefold: (i) the rearrangement of the constituents of the cell-body which is due to and indeed constitutes the actual process of dying—these are the sub-mortem[1] changes; (ii) the changes in the stored products of the cell activities which may occur sub-mortem, or only post-mortem as a result of the action of reagents; for instance, the swelling of mucin granules and consequent distortion of the cell-protoplasm, or the solution of the oxyphil granules of the coarsely granular blood cells of the crayfish; (iii) the action of fixing reagents in coagulating the cell-substance.

Of these three possible sources of artifact the last mentioned is dealt with first. The subject-matter of the paper therefore falls into three sections, which deal with (i) the nature of the structure produced in solid and liquid colloids by the action of fixatives, (ii) the production of structure by sub-mortem and post-mortem changes in the animal cell, and (iii) the application of the facts and principles set forth in the first two sections to the interpretation of structure seen in the cell-substance (α) when dead or dying, but unfixed (β), after fixation.

I take this opportunity, at the close of the introductory section, to express my sincere thanks to my friend Mr Neville, of Sidney Sussex College, for the unwearied patience with which he has aided me in these investigations. I have received both suggestions and criticism from him; indeed without his help, so generously given, the sections on the nature of the colloidal state could not have been written.

I. The nature of the changes produced in colloids by fixatives.

It is, I think, one of the most remarkable facts in the history of biological science that the urgency and priority of this question should have appealed to so few minds. Yet the urgency lies patent to the most superficial consideration. It is notorious that the various fixing reagents are coagulants of organic colloids and that they produce

[1] This phrase conveniently indicates that broad zone which intervenes between the completely living and the completely dead—the period μεταξὺ τοῦ θανάτου.

precipitates which have a certain figure or structure. It can also readily be shown, as will appear more fully in the sequel, that the figure varies, other things being equal, according to the reagent used. It is therefore cause for suspicion when one finds that particular structures which are indubitably present in preparations are only found in cells fixed with certain reagents, used either alone, or in particular formulæ. Altmann demonstrates his granules by the aid of an intensely acid and oxidising mixture, while Martin Heidenhain is dependent almost exclusively upon corrosive sublimate.

Though many writers deal episodically with the action of fixing reagents, the matter has received special attention at the hands of, so far as I know, only four workers. These are the three botanists Berthold[1] (1886), Fr. Schwarz[2] (1887) and Fischer[3] (1894—5) and the zoologist Bütschli[4] (1892). These workers are singular in the fact that they attacked the problem from without; they endeavoured to determine the nature of the action of fixing reagents on cell-substance by control experiments made on material such as solutions of gelatine or peptone, the physical homogeneity of which can be taken for granted.

Berthold added little to what had been previously said by Flemming. He criticised the net which Schmitz described in *Bryopsis* and *Saprolegnia* preserved with picric acid and roundly styles it an artificial product. He supports this assertion by experiments which prove that when egg-white is precipitated it takes a most beautiful "framework" structure. In the next year Fr. Schwarz in the course of a long treatise on the morphological and chemical composition of protoplasm, stated that the examination of living and fixed vegetable cells led him to the conclusion that the cell-protoplasm does not contain a "preformed" net or framework (Gerüst). This conclusion he supports by experiments on solutions of gelatine, peptone and dried egg-albumen, which showed that when these solutions are fixed by various reagents, they exhibit all the various appearances, granular, fibrillar and fine and coarse nets, which are to be seen in fixed cell-substance.

Fischer attacked the subject quite independently, and added two important observations to those made by Berthold and Fr. Schwarz.

[1] *Studien über Protoplasmamechanik.* Leipzig, 1886, p. 62.
[2] Cohn's *Beiträge zur Biol. d. Pflanzen*, v. p. 1. 1887.
[3] *Anat. Anzeiger*, IX. p. 678, 1894; X. p. 769. 1895.
[4] *Protoplasm and Microscopic Foams.* London, 1894.

The object of his experiments in the first instance was to determine whether Altmann's granules are or are not artifacts due to the action of the reagents. The result of his experiments proved, however, so striking as to lead him to condemn as artifacts not only Altmann's granules, but also the "tingible bodies" of Flemming, and possibly the whole of those appearances in the nucleus and cell-body grouped by Flemming under the heading "chromatolysis."

Fischer used solutions of peptone (2—10 %), hæmoglobin, nuclein, and various proteids such as serum albumin, paraglobulin, etc., either alone or mixed. He found, like Berthold and Schwarz, that the precipitates produced by various fixing reagents (osmic acid, chromic acid, Flemming's and Altmann's mixtures, etc.) had a certain figure—granular or net-like. He also showed that this is true, even when the solution is enclosed in thin solid membranes. Small pieces of elder pith were infiltrated with 2 to 10 % peptone solution, and then fixed in 1 % osmic acid, or in Altmann's mixture. Sections were made, and they showed the contents of the pith spaces arranged so as to offer a picture remarkably similar to that shown by sections of an actual cell. "In the middle was a nucleus-like body, from all sides of which there stretched to the cell-wall beautiful delicate threads which anastomosed with one another, and were composed of small and large granules." The second important point established by Fischer was the fact that when mixtures of colloids are used, such as peptone and serum albumin, the constituents could be "differentiated" by fixing and staining. In the case quoted, after fixation and staining by Altmann's method, the serum albumen was found to form a matrix holding peptone granules.

The importance of this last observation can scarcely be overrated. Solutions of peptone and serum albumen are miscible in all degrees, so there can be no question of the completeness with which the two substances are intermixed at the commencement of the experiment. Therefore it is clear that the fixing agent not only separates both dissolved substances from the water, but also separates the one from the other, so that a structure is obtained differentiated both in form and in staining reaction which bears no resemblance whatever, and gives no clue to the relation of the three constituents, water, albumen and peptone, before the fixing agent acts.

Bütschli's observations are especially interesting, not from the interpretation he places on them, because in this he has, I think, fallen into error, but from his clear recognition of the extreme importance

of the facts. In the text of the book he is disposed to deal somewhat summarily with the view that the appearance in fixed cell-substance is an artifact. Some investigations which he carried out himself led him completely to alter this attitude, and the changed view finds expression in the appendix. There he confesses that coagulated white of egg or commercial gelatine show all the appearances which are presented by fixed cell-protoplasm; those appearances which Bütschli interprets as indications of a very fine emulsion, or foam, structure. Ardent advocate as he is for structure in cell-protoplasm, Bütschli is compelled to admit that this fact deprives observations upon fixed cells of any evidential value. But, he says, the view held by Berthold, Schwarz and Kölliker, that the structures which are alleged to be present in protoplasm are really simply due to processes of coagulation of this kind, is untenable "in view of the numerous cases in which the structures are distinctly demonstrable in living protoplasm." The value of the last-mentioned evidence has, it seems to me, been very much overrated. This point, however, I hope to take up in a future paper.

My own work leads me to think that the action of fixing agents may be viewed in a much more comprehensive way.

I would start the discussion with no statement as to the nature of cell-protoplasm other than that it is, as Dujardin described it, "glutinous." Now this glutinous character is a special characteristic of that state of matter to which Graham applied the word "colloidal." If then we amplify Dujardin's adjective so far as to define living matter as matter either in or not far removed from the colloidal phase, we shall be within the limits of absolutely assured knowledge. But living matter does not lose its colloidal characters when it dies slowly, or when it is killed quickly by the action of some fixing reagent. The colloid substance of a cell does not become crystalloid as a result of the action of, *e.g.*, mercuric chloride. But, though it does not become crystalloid, the action of the fixing reagent is such as to produce an insoluble modification of the colloidal matter. Now the question which concerns us is, by what internal rearrangement of the solid and liquid constituents is this modification brought about?

My own experiments furnish the following general answer to that question. In the formation of an insoluble modification of a colloid from a soluble form, there is a separation of the solid from the liquid, so that the particles of the former adhere to form a framework which holds the liquid in its interstices.

This statement holds without modification whether the initial stage, that is the soluble colloid, be entirely fluid (colloidal solution), entirely solid (jelly), or a mixture of the two; or whether the physical change is or is not accompanied by chemical change. Lastly, the figure which the framework offers depends primarily upon the concentration of the initial stage, *i.e.* the proportion of solid to liquid, and upon the nature of the fixing agent which is used, and upon the particular colloid employed, and to a less extent upon the temperature during and subsequent to fixation, and the nature and concentration of the crystalloid bodies not immediately concerned in fixation, which may be present during the process.

Among fixatives I would include heat, and, in addition to this, I used osmic acid, as a 1 % solution, and vapour; potassium bichromate, 1 %; corrosive sublimate solution saturated in 0·6 % sodium chloride; formaline and potassium sulphocyanate. This list offers examples of fixing agents of both the slowly and the quickly acting types.

Two very different experimental methods were used to determine the condition of the material—these were direct microscopical examination, and what may be called pressure experiments.

The first consisted in the examination of films, preparations made by teasing, and sections prepared with the freezing microtome, and, after embedding in paraffin, with the rocking microtome. The preparations were examined in air, water, alcohol, or canada balsam. For extreme magnification, I used an admirable Zeiss apochromatic objective with angular aperture 1·40 and a focal length of 2 mm. Sections cut in paraffin were usually cut with only one tooth of a rocking microtome. Such sections vary in thickness from 0·6 to 1 μ.

In a fair number of cases, notably after osmic vapour, sections even thinner than this were made—that is to say, the wheel of the microtome was moved less than one tooth.

The pressure experiments need a word of introduction. It occurred to me that if, as microscopical examination seemed to show, all insoluble gels[1] have an open sponge structure, then one ought to be able to express from them the fluid contained in their meshes by comparatively small pressures, which should bear some very general relation to the size of the meshes.

[1] Graham's nomenclature is as follows: The fluid state, colloidal solution, is the "sol," the solid state the "gel." The fluid constituent is indicated by a prefix. Thus an aqueous solution of gelatine is a "hydrosol," and on setting it becomes a "hydrogel." There are etherogels, sulphogels, etc., which contain respectively ether and sulphuric acid.

The statement that the pressure necessary to express the fluid varies in some inverse measure of the size of the meshes of the sponge follows from Poisseuelle's law for the outflow from capillary tubes. Guerout made a similar use of this law to obtain a first approximate measure of the diameter of the pores in bladder, gold-beater's skin, and parchment paper[1]. Pressure experiments also enable us to discriminate between an open sponge structure and a vesicular or honeycomb structure. The pressure required to separate the fluid being greater in the latter than in the former case, when the dimensions of the structures are approximately the same.

The pressure required to separate fluid and solid from a solid structure containing both will depend upon the nature of the solid, and the size, shape and relative disposition of the fluid-holding spaces. Information on these points was desired for the purpose of these experiments. The pressure, however, also depends upon other relations, namely, the presence or absence of a continuous external limiting membrane of impervious solid, the viscosity of the fluid, and the cohesion between fluid and solid. The influence of any limiting solid membrane would be very difficult to estimate[2]. It may, however, be eliminated by cutting the mass into pieces. The viscosity of the expressed fluid did not vary in the different cases sufficiently to contribute much to the difference of pressure.

Actual experiment showed that fixation of any colloidal mass involves a change in the relations of solid and liquid, so that the latter can be more readily expressed. Thus a hydrogel containing 13 %[3] pure gelatine at a temperature of 15° will endure a pressure of 400 lbs. to the square inch—this is the maximal pressure I was able to employ—without separation of fluid. After fixation with formaline or corrosive sublimate, the solid and fluid are so much separated from one another as to permit of the latter being squeezed out, as from a sponge, with simple hand-pressure.

The phrase "insoluble gels" needs definition. Gels, such as a jelly of agar, of silica, gelatine, and celloidin are mixtures of solid and liquid. Of these mixtures I distinguish two classes which can be discriminated by the action of changes of temperature. They may be styled the gelatine class and the silica class after the best known examples; members of the former class are rendered more fluid by a rise of temperature; while members of the

[1] *Ladenburg*, III. p. 299.

[2] Cf. Osborne Reynolds on "Dilatancy," *Nature*, XXXIII. p. 430. 1885—6.

[3] Here and elsewhere this means grams per 100 c.c.

latter class become less fluid under the influence of the same change. Thus hydrogels of gelatine are mobilised by heat, they absorb water at a more rapid rate, and at a certain point, determined in the main by the proportion of solid and liquid, they become hydrosols. A hydrogel of silicic acid, of many metallic hydrates and sulphates, or of gelatine after it has been exposed to the action of formaline loses its power of holding water when the temperature is raised. To use Graham's terms, the "clot" shrinks and "serum" is expressed.

For the purpose of a purely histological treatise, and without going too deeply into the physical problems involved, these gels of the second class may be defined as those in which the solid constituent is insoluble in the fluid constituent, and this is what is meant by the words "insoluble gel" when they are used in the text. The words also imply that the gels in question are not solid solutions. This is proved by the fact that the curves of the rate of evaporation, and of the relation of the vapour pressure to concentration, are bending lines and not straight lines[1].

Physicists, notably van Bemmelen, have arrived at the conclusion that certain of the properties of gels of silicic acid, cupric hydrate, etc., which belong to my insoluble class, can be best explained by supposing that they have a sponge structure, being built up of a more solid framework holding fluid in the interstices. My own investigations have shown that this view can be confirmed by the microscope—the framework can be detected with, in some cases, even low magnification.

The great value of the pressure experiments lies in the fact that they supplement and verify microscopical observations, in one particular wherein the latter is notoriously weak. Sections of cells or of more simple colloidal masses, can furnish positive evidence of the existence of a framework; but they yield only unsatisfactory negative evidence as to the condition and nature of the material which occupies the meshes, unless indeed that substance can be differentiated by stains. In by far the majority of cases it is impossible to stain matter in the meshes of the net: this however would scarcely serve as an adequate reason for believing that the meshes are occupied by a simple fluid, the "serum" as Graham styled it of the coagulated mass, seeing that the cell histologist speaks freely of the existence in the cell of "non-staining" substances. Pressure experiments therefore serve most opportunely in affording proof that "non-staining" substances means

[1] Spring and Lucion, *Zeits. f. anorg. Chem.* II. p. 195. 1892. Tamman, *Zeits. f. phys. Chem.* X. p. 263. 1893. v. Bemmelen, *Zeits. f. anorg. Chem.* V. p. 467. 1893, and XIII. p. 233. 1896.

for the most part simple spaces occupied only by a fluid which varies according to the menstruum in which the coagulated colloid mass or the tissue may chance to be lying[1].

It will simplify the method of treatment if I state at the outset the significance to be attached to the remark wherever made that:— it was found impossible to demonstrate the existence of any material in the meshes of a net. This means that the following separate methods failed to drive stain into any substance in the meshes: the iron-hæmatoxylin method, used without any washing out soever; staining with saturated solution of acid and basic dyes at various temperatures; and evaporation to dryness in such solutions with or without subsequent heating.

Fixation of colloidal solutions. White of egg was broken up thoroughly, diluted and filtered. Solutions varying in concentration were made from the filtrate either by partial evaporation, or by evaporation to dryness and resolution. Tiny droplets, about 1 to 2 mm. in diameter, held in loops of silk thread were fixed in various ways and sections cut. Larger drops and masses of the solution in moulds of porous paper of different sizes were also fixed in the same reagents. The following stains were used: iron-hæmatoxylin alone and followed by osmic, methylene blue, eosine, and Ehrlich-Biondi's triple stain.

The following facts appeared to be established. 13 % solution fixed with corrosive sublimate sections made after embedding in paraffin were found to show a sponge or net structure, the aspect of which is given in Figure 1. It proved to be impossible to stain any substance in the meshes of the net. They contained simple fluid, of which direct hand-pressure[2] or a centrifugal machine expressed a quantity equal to ± 60 % by volume of the entire gel.

This net structure, visible under the microscope, might be due not to the action of the fixative but to heat shrinkage. This is not the case. It is formed in the process of fixation. The net can be demonstrated in films of the solution fixed by immersion in sublimate, in preparation from the fixed droplets made by teasing, and in sections cut from the gel immediately after fixing and washing in water by the freezing microtome.

[1] One becomes peculiarly sceptical as to the existence of colloid masses which cannot be infiltrated with some dye when one remembers that, as Graham and Voightländer (*Zeits. fur physik. Chem.* III. p. 316. 1889) have shown, salts diffuse through gels with so little hindrance that the rate is the same as in pure water.

[2] That is, pressure applied by a glass rod to the substance when in a glass vessel.

The effect of a rise of temperature upon the fixed colloid is complicated. If the heat is applied while the coagulum is in its original fluid, or in water, it behaves as insoluble gels commonly do—it shrinks. The rise of temperature leads to a closer aggregation of the solid particles—a synæresis, as Graham called it. When the original fluid has been replaced by such a fluid as xylol a rise of temperature produces a very small effect, but the process of dehydration itself causes a shrinkage amounting to ± 30 % of the original volume[1]. The initial coagulation, or fixing, is not necessarily or perhaps usually accompanied by a change of volume. This is an agreement with the fact first established by Graham that an insoluble gel when first formed has the same volume as the solution from which it is produced.

This statement holds good not only of simple colloids but also of such tissues as were experimented with, and the figures for shrinkage are about the same. Pancreas and liver of the frog were examined. I subjoin one example.

A strip of liver was cut out with fine scissors and when suspended it was found to measure 11 mm. by ± 2 mm.

Suspended in the sat. solution of sublimate in 0·6 sodium chloride.

After 2 hours length = 11 mm.
„ 24 „ „ = 11 mm. } no diminution of length.

Brought into 95 % spirit, containing a trace of iodine: after 12 hours length = 9 mm. = decrease in length of 18 %.

Further 24 hours in 95 % spirit = 9 mm.
20 hours in cedar oil = 9 mm. } no further shortening.

If the decrease were the same, namely 18 %, in all directions then the diminution in volume would be about 50 %.

The average linear shrinkage for strips of liver and pancreas was found to be nothing on fixing, and about 20 % on dehydration.

We may set these facts, the absence of any change of volume when the insoluble gel is formed, and the diminution of volume which follows when it is infiltrated with various fluids, or warmed, beside Graham's account[2] of the volume change of a gel of silicic acid—the type of insoluble gels—when its water is replaced by other fluids.

[1] It should be remembered that a change of 30 % in cubic capacity implies a very much smaller diminution in any linear measurement.

[2] *Journ. of the Chemical Society*, II. p. 318. 1864.

When a hydrosol of silica changes into a hydrogel there is no change of volume. But when the water in the sponge of the hydrogel is replaced by other fluids there is a decrease in volume. Alcohol and glycerine produce only a slight diminution of volume; sulphuric acid however leads to a diminution equal to one-fifth or one-sixth of the original volume.

I have determined the existence of a solid framework having an open net structure in the following gels. In white of egg coagulated by corrosive sublimate, heat, potassium bichromate, or a trace of potassium sulpho-cyanate. In a hydrogel of silicic acid, and in gelatine coagulated by sublimate, ammonium bichromate, or formaline. In a gel of celloidine produced by the action of chloroform on an ether-absolute solution, and lastly in common black indiarubber.

The last-mentioned case is interesting. Indiarubber is produced by the coagulation of an organic fluid, and it behaves like an insoluble colloid—it shrinks on warming and extends on cooling. It is therefore interesting to find that sections of black indiarubber cut with the freezing microtome show the characteristic structure, namely a fine open sponge.

The sponge structure of these insoluble gels, which is here demonstrated by direct observation, has been inferred by physicists from their examination of the relations of fluid and solid in such gels. Thus, to quote only the chief worker in the field, van Bemmelen[1], who constructed curves of the removal and absorption of water for various gels comes to the conclusion that the process of coagulation by which these gels are formed consists essentially of a separation of solid from liquid; the solid particles then hang together to form a framework which encloses the separated solvent.

In other words, and this is what I wish to insist upon here, the very essence of the process of fixation is the separation of solid from liquid and the formation thereby of a structure which may have had no counterpart whatever before fixation occurred.

I turn now to consider the various factors which modify the configuration of this structure.

The influence of the nature of the fixative, and the nature and concentration of the colloidal solid upon the final configuration. The

[1] *Berichte d. deut. chem. Gesell.* XIII. p. 1467. 1880; *Journ. f. prakt. Chem.* XXIII. pp. 324 and 379. 1881; *ibid.* XLVI. p. 497. 1892; *Zeits. f. anorg. Chem.* V. p. 467. 1893; *ibid.* XIII. p. 233. 1896.

relations which fall under this heading are fairly simple for solutions of egg-white, but very complex for solutions of gelatine.

Egg-white. The figure is always that of an open net with spherical masses at the nodal points except when the fixative is osmic vapour. The size of the nodal spherules and dimension of the meshes of the net vary according to the nature of the fixative and the concentration of the egg-white solution.

The procedure in all cases was as follows. The droplets of the solution of egg-white were suspended in the fixative in loops of silk. They were exposed to the sublimate solution for 20 hours; to osmic vapour for 12 hours in the dark; to potassium bichromate, a 1 % solution, for 21 days. To fix by heat the droplets were held for a moment until opaque white in a jet of steam. After fixation the droplets were washed in distilled water for about six hours, and dehydrated by changes of spirit, 15 %, 30 %, 40 %, 50 %, and so on; and imbedded in paraffin from cedar oil.

Fixation by potassium sulphocyanate needs special mention. Meusel in 1886[1] described the coagulative action of this salt in a brief paper which does little more than record the experiments. He found that when 8 grams of the dry salt are added to 30 c.c. of white of egg or serum the latter sets to a firm jelly in the course of a few days. As the original paper is bare of detail I subjoin an account of one of my own experiments.

White of egg beaten and centrifugalised: solids in clear fluid determined and found to be 13 grams in 100 c.c.; to 30 c.c. of the clear fluid 8 grams KCyS were added; the salt dissolved with great rapidity and without any clouding; placed in a moist chamber—temp. $\pm$ 16°.

24 hours still fluid and clear.
48 „ becoming viscid and slightly cloudy.
72 „ much more viscid and still more cloudy.
96 „ set to an opaque white jelly; not very firm.
Later jelly quite firm and is contracting slowly with expression of a clear serum.

The jelly is insoluble in distilled water even after the sulphocyanate has been allowed to diffuse out into several changes of distilled water.

I found however that it is not necessary to employ such a large quantity of the salt; coagulation can be effected by a small trace only if the temperature be raised. Thus: 5 c.c. clear solution of egg-white

[1] Dr Ed. Meusel. *Die Quellkraft d. Rhodanate*, 1886.

holding 10 % solids was warmed to 45° and a minute crystal of KCyS added. In 1½ hours it had set completely to a slightly opaque jelly.

Films were also fixed by traces of the salt in a warm moist chamber. In all cases a delicate net structure was demonstrable. These details are needed since these gels were used to investigate the action of ordinary fixing reagents on already formed "insoluble" gels.

The effect of the nature of the fixative is shown by the following table, which gives the average value for the length of a diagonal of the mesh when the amount of coagulable solid in the original solution was ± 13 grams in 100 c.c.

osmic vapour	0·5 to 0·7 μ
steam	1 μ
potassium sulphocyanate	1·0 μ
potassium bichromate	1.3 μ
corrosive sublimate	1·7 μ

It will be seen from the marked difference between the figure for sublimate and that of the rest that in the fixation of proteids this reagent has, as Henneguy remarks, "a certain brutality of action" which unfits it for use in the study of the structure of protoplasm[1].

The configuration after fixation by osmic vapour appears to be different from that produced by the other reagents. It looks to be that of a number of vesicles hollowed out of a continuous solid mass and therefore not communicating with one another. It is however very difficult to be certain on this point.

The effect of the concentration of the solution of the egg-white when it is above the minimum which is necessary for the production of a continuous mass in the fixed state is restricted to an alteration in the size of the meshes. Figures 1, 2 and 3 show the appearance of the net produced by sublimate in solutions of egg-white containing respectively 13, 30 and 60 grams solid per 100 c.c. The microscopical analysis of the figure in the last case is a very doubtful matter. When the microscope is pushed to these limits its powers are more potent in magnifying the personal equation of the observer than in really

[1] *Leçons sur la cellule*, pp. 42 and 61. Henneguy is so impressed by this "brutality" as to lead him to reject the observations of the important school of Louvain (Carnoy) owing to the fact that the workers rely almost exclusively on this fixative (p. 42). He makes the interesting remark that when one looks through the plates in "La Cellule" one is struck by "l'uniformité de structure que revêtiraient les cellules les plus diverses," and he asks "si celle-ci n'est point due à l'uniformité de la méthode employée."

elucidating details of structure, seeing that the structures under observation become commensurate with the diffraction areas. However it is certain that there are discontinuities—the doubt is limited to whether the open net structure is still retained, or whether the spaces are discontinuous in the form of separate vesicles.

If the concentration of the colloidal solid is very small a granular precipitate may be produced. The particular concentration necessary for the production of a continuous net varies with the rate of fixation, the absence of mechanical disturbance, and, as we shall see later, the concentration and nature of any electrolytes which may be present. The continuous net and the precipitate are not discontinuous states. They pass into one another with variations in concentration.

With egg-white when the concentration is 10 grams per 100 c.c. and the fixative potassium sulphocyanate the figure is a continuous open net. When the concentration is lowered to 6 grams per 100 c.c. a double structure is visible, the typical fine net is no longer continuous throughout the entire mass. Clefts and spaces appear in it so that sections have the aspect of a very coarse sponge the bars of which are built out of the typical fine net. With still further dilution the fine net is formed in completely discontinuous patches which constitute a "flaky" precipitate. This flaky precipitate therefore is formed as it were by the rupture of the coarse framework mentioned above. Finally, with yet greater dilution, the fine net is not formed even in patches, but in its place there appears a suspension of discrete spherical granules.

Gelatine. The figure in this case changes not only in dimensions but in character. If gelatine of medium concentration, say 7 to 15 grams solid to the 100 c.c. be fixed by alcohol, or sublimate, instead of forming an open net, it takes the form of a continuous solid hollowed out by vesicles about 7 μ in diameter (Fig. 6) which as a rule become deformed to polyhedra by mutual pressure. If it be fixed by formaline however an open net is produced; the formaline however must be in excess and be allowed to act on the gelatine for about 16 hours. The open net figure may be formed with alcohol or sublimate but in that case, supposing the reagent to be present in large excess, the percentage of gelatine in grams per 100 c.c. must be less than 5. The figure then consists of nodal spherules joined by bars as when white of egg is fixed (Fig. 5). The dimensions of the meshes and spaces are as follows:

Fixative sublimate: sat. sol. in 0·6 % NaCl.

grams gelatine per 100 c.c.	Figure	Dimensions
4	open net	diagonal of mesh 2 μ
10	honeycomb	diameter of sphere 7 μ
25	,,	,, ,, ,, 3 μ
50	,,	,, ,, ,, 2·5 μ

Figures 5 to 8 illustrate these cases.

The point at which the figure changes from the open net to the honeycomb depends not only upon the concentration of the colloidal solid but also upon the proportion of alcohol or sublimate in the changing system. I have not followed this point in detail though it is of considerable theoretical importance. The following instance will serve to prove the general truth of the statement :

$\frac{\text{vol. alcohol}}{\text{vol. } H_2O}$	grams gelatine per 100 c.c. necessary to effect the change from the open net to the honeycomb
1	$\pm$ 20
∞	4—5

These involved relations depend upon the fact that sublimate and alcohol are largely miscible with gelatine in presence of water, and they thus form with the water and gelatine a ternary system of partially miscible substances. The sublimate and alcohol do not destroy the gelatine-water system as does *e.g.* formaline, they only modify it. Thus one volume of a 50 % solution of gelatine mixes completely with one volume of a saturated solution of sublimate, forming a system which, like the original gelatine-water system, is heat-reversible, being fluid at above 50° and a transparent jelly below.

We must therefore distinguish two cases, (i) the production of a true insoluble modification, and therefore the complete destruction of the original system, as when formaline acts fully upon gelatine, or when sublimate acts upon egg-white; and (ii) the modification of the original system so that though altered it still remains within the class of soluble colloids—becoming fluid on heating in presence of a certain quantity of water, and solid on cooling. The final figure appears to be in the former case always that of an open net if the concentration of the colloidal solid be above a certain minimum; in the latter case when the fixative is in excess it is a honeycomb unless the concentration of the colloidal solid be very low.

Fixation of a soluble gel. Fixatives produce the same general effect, namely, a separation of a more fluid from a more solid part, whether the soluble colloid is exposed to them in the solid state or the fluid state. This was determined by comparing the results of fixation when both the mixture of gelatine and water and the fixing solution were above the temperature of melting of the former, and when they were below that temperature. The internal change in the jelly is manifested clearly by the effects of pressure. A hydrogel of gelatine holding ± 13 grams solid in 100 c.c. will endure a pressure of ± 400 lbs. to the square inch without separation of fluid. Fixation with formaline produces little superficial change. The jelly becomes firmer but retains its transparency. Its internal structure is however changed to such an extent that fluid can be expressed by hand-pressure alone.

The absence of any obvious optical changes during the process of fixation is remarkably evident in the fixation of an ether-alcohol solution of celloidin by chloroform. A clear transparent solid is produced. The meshes however of this gel are so coarse that the greater part of the contained fluid can be wrung out with the fingers as though the mass were a common sponge.

The theory of the changes which are produced by fixatives[1].

The facts which are stated empirically in the foregoing sections are susceptible of rational treatment to a limited extent. Put briefly the facts show that when a soluble colloid is fixed by the action of a fixing reagent it acquires a comparatively coarse structure in the process which differs wholly or in part from the structure of the soluble colloid. It is possible to make a general statement of the relation between the initial and final states in this change.

On page 165 I drew attention to the fact that colloidal mixtures[2] differ from one another in their physical characters, and I distinguished between two classes. To this point it is necessary to return.

Fluid mixtures of water and *e.g.* agar, gelatine, proteid, silicic acid, aluminium hydrate, or antimony hydrosulphide; or of absolute alcohol and celloidin form binary systems which are capable of passing into the solid state known as the gel. In some cases the change of state follows

[1] The purely physical conceptions, and the experimental results which are briefly stated in this section, will be more fully stated elsewhere.

[2] Colloidal mixtures can be classified but not individuals, for the same individual will form a mixture of one order with one solvent, of another order with another solvent.

on a simple fall or a simple rise of temperature, in other cases it occurs only when the primary system is modified by the introduction of some reagent, and in the process the colloidal solid may suffer chemical change. No matter how it is accomplished the change to the gel state enables us to place the systems in two groups; in the first the change of state is reversible by altering the sign of the temperature change—*e.g.* gelatine or agar and water; in the second the change of state is irreversible, permanent molecular aggregates being formed. We will consider first the former, the heat-reversible systems, or soluble colloids as they are called in the preceding pages.

Heat-reversible colloidal mixtures. Up to the present nothing has been known and but little even conjectured as to the relation of such mixtures as gelatine-water, or agar and water to other states of matter. I have, I think, obtained experimental proof that they may be regarded as special cases of partially miscible fluids.

Agar-agar and water was the mixture experimented with. It was prepared free from diffusible bodies. By small pressure a jelly holding 1 gram agar in 100 c.c. may be separated into two parts; (*a*) a solution of water in agar, (*b*) a solution of agar in water. The former is at 15° a solid solution. These two solutions form a binary system of partially miscible solutions—that is to say they are completely miscible above a certain temperature, in which state a homogeneous fluid is formed which is a solution of agar in water. Below a certain temperature the homogeneous liquid divides into a pair of liquids which are separated by a surface across which diffusion occurs. Such a pair of liquids have been called conjugate liquids[1].

With still falling temperature one phase of the pair—the solution of water in agar—becomes a solid solution, the solid phase forming first as a membrane at the surface. This change is the cause of the change in the whole system whereby it sets to a jelly. This agar-water system therefore resembles a mixture of for instance phenol and water. Above 80° phenol and water are miscible in all proportions; below that temperature if more than 71 or less than 76 % of phenol is present the mixture forms a pair of conjugates, one a solution of phenol in water, the other a solution of water in phenol. What is known of these mixtures is almost entirely due to the work of Alexejeff[2]. The chief properties which have been determined for partially miscible liquids

[1] Neville. *Science Progress*, IV. p. 77. 1895.

[2] *Annalen der Physik und Chemie*, N.F. XXVIII. p. 305.

are that the composition of the two phases of the conjugate is independent of the total composition of the entire mixture, but is determined by temperature. These two relations hold for agar-water. In a mixture holding 3·5 % (grams agar per 100 c.c.), at 15° the solution of agar in water phase contained 0·45 % agar; while in a mixture holding 1 % agar at the same temperature the same phase also had 0·45 % agar. On the other hand the percentage composition of the two phases was found to vary with the temperature. Thus, taking the same phase of a mixture holding 2·5 % agar the figures obtained were

°C.	%
± 1	0·35
10·5	0·42
32	1·00
42	1·66

The difference between the agar-water system and any partially miscible fluids hitherto investigated lies in the contour of the surface of contact of the conjugates. In phenol-water, ether-water, or any such systems the surface is a plane—that is to say, it is minimal. In the agar-water system it is curved and discontinuous, so that when the conjugates form, one phase separates as droplets immersed in the other. It will, I think, be possible to refer this peculiar feature to the very great difference in the mobility of the molecules of the two constituents of the mixture—the agar and the water. Owing to this feature the system attains true equilibrium very slowly indeed—in the case of *e.g.* gelatine holding 2·5 % solids only after many months.

Apart from this difference a mixture of agar and water closely resembles a mixture of salicylic acid and water[1]. Salicylic acid has a high melting point (151°), but when heated under water to 100° it melts, forming a fluid hydrate which becomes miscible with water at 106°. On cooling this mixture it separates into conjugates. Thus this substance, like agar, is solid at ordinary temperatures but forms a fluid hydrate which in turn forms a partially miscible system with water.

The actual configuration of these soluble jellies can be studied with even low powers of the microscope by using ternary instead of binary mixtures. Alcohol, pure gelatine[2], and water form such a ternary mixture. If 13·5 grams gelatine are mixed with 50 c.c. H_2O and 50 c.c. absolute alcohol, one has a mixture which is homogeneous at about 17°,

[1] Alexejeff. *loc. cit.*

[2] That is gelatine freed from diffusible substances by washing with distilled water for some months.

but which forms a pair of conjugates below this temperature. The changes on cooling as seen on the stage of the microscope are these—when the temperature reaches 17° small droplets appear in the homogeneous fluid which attain a maximal size of 3μ. The droplets are at first separate and fluid; owing to their presence the viscosity of the mixture very much increases. As temperature falls the droplets adhere to one another, forming a framework which at 12° is composed of a solid solution composed of spherical masses hanging together to form anastomosing threads. The droplets slightly deform each other where they touch. At this stage the entire mass forms a jelly. At 14° the distribution of gelatine in the two phases is as follows:—100 c.c. of the droplets holds 18 grams gelatine; 100 c.c. of the nexus fluid holds 5·5 grams gelatine.

Ternary mixtures have been studied only by Duclaux[1]. They are formed most readily by converting a pair of immiscible fluids into partially miscible fluids by the addition of a third substance, which dissolves both. His figures, as Ostwald points out[2], show that these mixtures behave in a simple manner resembling a binary system if the solubility of the two immiscible substances in the common solvent is approximately the same. If the solubility is very different then the relations are complex. A mixture of benzene, acetic acid and water is a system of this kind. The common solvent is the acetic acid, but whereas water and acetic acid readily mix with rise of temperature, benzene and acetic acid only mix freely at temperature above 15°. In this case the distribution of the constituents in the conjugates varies widely with variations in the composition of the whole mass.

Gelatine, water and alcohol form a ternary mixture of this type. The common solvent is water—it mixes freely and in all proportions with alcohol with liberation of heat: it is only completely miscible with gelatine at temperatures above ± 40°. Therefore one finds that the distribution of the constituents in the two phases is much altered by changes in their relative proportions in the entire mass. So far I have only studied the effect of changes in the amount of gelatine on the distribution of this substance. Stated in grams in 100 c.c. the figures are as follows:—

	mixture	droplets	nexus fluid
$T = 15°$	6·7	17	2
	13·5	18	5·5
	36·5	8·5	± 40

[1] *Ann. Chem. Phys.* (5) II. p. 264. 1876.

[2] *Lehrbuch d. allgem. Chemie*, 2nd Ed. Vol. I. p. 821. 1891.

These figures illustrate a remarkable property of these mixtures which appears to hold whether they are binary or ternary mixtures. In the particular case under consideration the gelatine is chiefly contained in the droplets up to a mixture holding 13·5 %; somewhere between this and 36·5 % the system becomes inverted[1]—the droplets now containing the smaller quantity of gelatine. Thus if the mixture with the lower concentration is heated until it is homogeneous and then allowed to cool it separates into droplets containing a large amount of gelatine which at a slightly lower temperature become solid solutions of water and alcohol in gelatine. Therefore in the jelly stage the framework is a solid net holding fluid in its interstices. In the case of the mixture with a higher gelatine content the configuration of the jelly is the exact converse. As cooling goes on in this case droplets separate with a small gelatine content, and it is the nexus fluid which passes into the stage of solid solution. The droplets in the latter case therefore are quite regular vesicles 10μ in diameter.

There are therefore theoretically three states in which these mixtures of water and solid forming the class of heat-reversible or soluble colloids can be, namely, (1) a homogeneous fluid; (2) a heterogeneous fluid, having the form of an emulsion of conjugate, as opposed to immiscible, fluids; (3) a pair of conjugates, one a fluid, the other a solid solution. In the third state the configuration as already pointed out differs remarkably according to concentration. The reaction of such a system to external agencies varies according to the state in which it happens to be; in the third state for instance, not only is there the interdiffusion between the two phases but also capillary phenomena are manifested. Mixtures of agar and water, gelatine and water, certain carbohydrates and water, such as in Lyle's golden syrup, are in one or the other state according to the relative amounts of the constituents present and the temperature. The conjugate state is reached at ordinary temperature in the two first-named mixtures, even when the concentration of water is very great; in the last case however the concentration of water must be small and the temperature low.

It is clear that of these states one, the solid or gel state, is of secondary importance—that is to say, it is dependent upon the formation of a membrane at the surface of separation of the conjugates, or upon the passage of one phase into the state of solid solution. This point may be made clear by an experiment which was suggested by my

[1] That is the major part of the gelatine changes from the concave to the convex side of the surface of separation.

friend Mr Neville. Ether and water form a pair of conjugates which are separated by a plane surface. If they are violently shaken an emulsion is produced which is however unstable and passes rapidly into the stable state in which the surface of contact is minimal. The emulsive stage may however be made permanent so that a jelly-like mass of adherent droplets is produced by the introduction into the system of a substance whose solubility in the two phases is very different, and which is a solid at ordinary temperatures. Thus if water is saturated with iodine and then shaken with ether a permanent emulsion is formed, the droplets of which adhere to form a jelly-like mass. On warming, the droplets fuse and the jelly stage vanishes. The phenomenon is due to the formation of a tenacious film of concentrated iodine at the surface of the droplets.

The phenomenon is related to the fact that iodine is solid at ordinary temperatures. Bromine is fluid at ordinary temperatures. It has, however, about the same relative solubility in the two phases as has iodine. In spite of this however an ether-water-bromine mixture made in the same way will not form a permanent emulsion, nor will it make a jelly. The iodine-water-ether mixture manifests a striking property of soluble colloids, namely that it forms very permanent foams. One might also point out in passing that the vapour-pressure of the iodine would be quite abnormally low. It would be that of the minute amount dissolved in the nexus fluid.

The formation of a surface film on droplets was observed by M. Traube[1] in a variety of cases, by Lehmann[2], and by myself in the case of ternary mixtures holding gelatine.

These facts and conclusions show that the class of heat-reversible colloidal mixtures may possibly include members which at no concentration form gels, though at certain temperatures and concentrations they may form conjugates.

One may perhaps regard the proteid-water system, or rather the egg-white-water system, as being one of these for the following reasons. So long as the changes of temperature are kept within those limits which do not destroy the system by producing chemical change, the changes of viscosity are of the same sign as those of aqueous solutions of agar or gelatine, and of the opposite sign to those of a solution of silica—that is to say, irreversible molecular aggregates are not formed on raising the temperature. The change in viscosity

[1] *Arch. Anat. u. Physiol.* pp. 83, 129. 1867.

[2] *Molekularphysik*, I. p. 508. Leipzig, 1888.

produced by varying the concentration of the egg-white differs from that produced by changes in the concentration in true solution. The only data are those furnished by Bottazzi[1]. His figures do not stand very close together but they show that the viscosity of proteid solutions does not change continuously with changes in concentration as it does in crystalloid solutions, but it changes *per saltum*. That is to say, there is a critical concentration marked by a sudden and extraordinarily great increase in viscosity above the values for lower concentrations. This cannot be due to change in the size of the molecular aggregates, for that would proceed continuously with change in concentration. It would however be produced by change from a homogeneous fluid to a pair of conjugates with a very large surface of contact[2].

Colloidal mixtures which form irreversible molecular aggregates when they pass into the gel state. These mixtures have been examined by a number of physicists. In the fluid state the relations of the solid and fluid are very far removed from those found in true solution, so far indeed as to render conclusions as to *e.g.* molecular weights, derived from the application of laws which are known to be true only for crystalloid solution in its most perfect form, that is when concentration is very small, worthless to the verge of ridicule. These colloidal solutions cannot be discriminated from non-settling suspensions of solid particles in a fluid medium such as those of gold in water which were prepared by Faraday, and are still preserved at the Royal Institution. The characteristic phenomena which they manifest are the following.

The molecular aggregates which form the dispersed particle of solid become larger by a process of clumping as a result of the presence of electrolytes—that is of salts; and the process is aided by a rise of temperature. The action of electrolytes in increasing the size of the molecular aggregates was worked out by Picton and Linder[3] with the aid of various optical methods including the microscope. If the concentration of the electrolyte is sufficient however the internal changes proceed beyond the simple increase in the size of the molecular aggregates to the actual solidification of the entire mass—that is to the

[1] *Arch. Italienne de Biol.* XXIX. p. 401. 1898.

[2] To safeguard myself from misconception I must point out that the viscosity of colloidal solutions is probably of different origin from that of the viscosity of crystalloid solutions. A clue to its nature is found in the experiments of Frankenheim (*Journ. f. prakt. Chem.* LIV. p. 433. 1851) on the influence of suspended particles upon fluid properties. I hope to discuss this and kindred points elsewhere.

[3] *Trans. Chem. Soc.* LXVII. p. 63. 1895.

point of coagulation of the mixture. It is obvious therefore that there exists some connection between the size of the particles and the process of coagulation, and Graham I believe somewhere suggests it. One obvious inference from the facts is that when the molecular aggregates attain a certain size the fluid condition is no longer possible; this would follow immediately from Graham's observation that actual coagulation is preceded by a continuous increase in the viscosity of the liquid. I have been able to follow the entire change from the hydrosol to the hydrogel under the microscope, so that it is possible now to give a connected account of the mechanism of coagulation. Before proceeding to this I may note in passing that the similarity of these colloidal solutions to simple suspensions, such as *e.g.* China clay in water, is borne out by the fact that electrolytes and temperature act on both alike, in making the discrete particles of solid larger by a process of aggregation[1].

The generic action of salts as coagulants, as distinguished from any specific chemical action, was first discovered by Graham, and has since been thoroughly investigated by Schulze[2], Prost[3], and Picton and Linder[4]. Putting the facts and conclusions reached by these workers together and adding my own observations the conditions which determine coagulation may be stated as follows:—

(1) The point at which coagulation occurs is determined by the concentration of the solid—*e.g.* silica in the colloidal mixture, the temperature, and the molecular concentration[5] and nature of the electrolytes which are present.

(2) The concentration necessary for coagulation is lowered by a rise of temperature, or by an electrolyte.

(3) The coagulative energy of electrolytes as measured by the number of gram-equivalents per litre necessary to produce coagulation is determined almost solely by the nature of the metal of the salt; and among the metals themselves it is determined by the valency of the metal. The coagulative energy of metals of different valency is approximately in the ratio

$$V' : V'' : V''' = x : x^2 : x^3$$

[1] Cf. Bodländer. *Neues Jahrbuch f. Mineralogie*, II. p. 147. 1893.
[2] *Journ. f. prakt. Chem.* XXV. p. 431. 1882; *ibid.* XXVII. 320. 1883.
[3] *Bull. d. l'Acad. Roy. d. Sci. d. Belg.* Ser. 3. XIV. p. 312. 1887.
[4] *loc. cit.*
[5] That is gram molecules per litre.

(4) If two electrolytes are present then the action is additive if the metals are of the same valency, and subtractive if of different valency. Therefore there is interference between *e.g.* salts of the form $R'Ac^n$ and $R''Ac^n$—the one "inhibits" the other.

These relations therefore are exceedingly definite and seem to hold for all colloidal mixtures which form irreversible aggregates; and they differ as widely as possible from the relations which govern alteration in the conditions of colloidal mixtures of the heat-reversible type[1].

Those colloidal mixtures which form irreversible molecular aggregates in the process of forming a gel may unhesitatingly be placed in the class we are considering together with the type examples—silicic acid and certain metallic hydrosulphides, or hydrates and water.

Such a mixture is the one which is produced by boiling a filtered solution of egg-white in water containing ± 1 gram solids per 100 c.c. Haycraft and Duggen[2] first pointed out that if white of egg be diluted by the addition of about 8 volumes of water and boiled it does not coagulate but merely "becomes a little milky." If calcium or barium chloride or sulphate of magnesium be added to the fluid after boiling it readily coagulates, forming a gel either at the temperature of the air or at some higher temperature determined chiefly by the amount of the salt added[3]. I have reinvestigated these phenomena and I find that this heat-modified solution of egg-white forms irreversible molecular aggregates which may or may not proceed to the stage of coagulation under those conditions which characterise the colloidal mixture of the silica-water class. The mixture coagulates when the molecular concentration of the electrolytes which are present reaches a certain point which, for a given temperature, is determined by the valency of the metal. It is in this particular case that I have followed the events in coagulation from the process of formation of larger and larger aggregates, at which stage Picton and Linder left it, to the actual formation of a firm gel. The events are easily seen under the microscope because the refractive index of the aggregates is very different from that of the fluid in which they lie and they rapidly grow to a relatively large size (0·75 to 1μ). The simplest method is the following. A thin thread of silk is dipped in some solution of a salt (*e.g.* a 2% solution of calcium chloride) and laid on a slide, over this is placed a coverslip, and the boiled solution of egg-white is allowed to fill the

[1] Cf. for example Pascheles. *Pflüger's Archiv*, LXXI. p. 333. 1898.

[2] *Brit. Med. Journ.* p. 167. 1890.

[3] Ringer. This *Journal*, XII. p. 378. 1891.

space. The coagulum may be watched as it gradually forms round the silk thread. A magnification of 500 diameters is sufficient. The fluid at first is free from visible particles. We know however from Picton and Linder's work that the heat by chemically altering the dissolved proteid has by a process of desolution, to use Lord Rayleigh's word, produced a suspension of particles having an average diameter commensurable with the mean wave-length of light. Under the influence of electrolytes these particles aggregate to larger and larger masses, so that one first sees near the silk thread a fine cloud, the particles in which grow in size until they form spherules having a maximum diameter of 0·75 to 1μ. They are now seen to be arranged in patterns forming an open net with regular polygonal meshes having diagonals as long as 6μ. The threads of the net are formed of contiguous spherules. This stage however is not one of equilibrium—the net shrinks, the meshes become smaller and the spherules apparently shift their points of attachment until, in place of being bounded by threads composed of several spherules, the image has the appearance of the typical fine net with spherules at the nodal points joined by tiny threads. Whether these joining-threads or bars have a real existence, or whether they are purely optical and the spherules actually touch one another it is impossible to say at present. One important point struck me, namely that when the particles are large enough to be clearly visible with a magnification of 500 diams. they do not show Brownian movement—in other words they are probably already in some way linked to one another.

This then is the process of coagulation. To complete what can be said concerning the mechanism, a few words would need to be devoted to the nature of the directive force which aggregates the particle to visible spherules and then links these together in a definite figure. This matter, however, will be dealt with in a paper on coagulation produced by electricity.

Action of reagents upon heat-reversible colloidal mixtures. This can best be considered by dealing first with gelatine. Mercuric chloride and alcohol have the same action. The latter, as we have already seen, forms a ternary mixture with gelatine and water which, at certain concentrations and within certain limits of temperature forms a pair of conjugate fluids. Sublimate, gelatine and water also form mixtures which are fluid above certain temperatures and which on cooling form a pair of conjugate fluids, one of which on still further cooling becomes a solid solution. To give an example, one part of a 50% solution of

gelatine mixed with twice its volume of a saturated aqueous solution of mercuric chloride is fluid above 50°, and as the temperature falls it undergoes the changes described above.

The figure of the conjugate fluids differs according to the concentration of the gelatine in the mixture. If the mixture of gelatine and water contains more than 4% of the former the droplets are fluid; and the figure in the gel stage is that of a solid mass with vesicles hollowed out of it. If the percentage is less the figure is the obverse—an open net of solid solution is formed having fluid in its spaces. The configuration is not destroyed when the water is replaced by alcohol and the sublimate dissolved out by iodine.

If the sublimate is dissolved out, as *e.g.* by an aqueous solution of sodium chloride, the coarse figure reverts to the much finer structure of the gelatine-water system.

Formaline, at any rate when in excess, has a different action. The gelatine is altered so that it becomes permanently and absolutely insoluble in water. The jelly which it now forms behaves like a jelly of silicic acid and water—it shrinks on warming. Its physical characters are unaltered by complete removal of any free formaline and exposure to distilled water for 10 months. The structure of this gel is that of an open net holding fluid in its interstices. I have found this figure in all the true insoluble gels which undergo shrinkage on warming which I have examined.

These are thus two extreme cases. In the first the primary system is modified in degree but not in kind by the introduction of a third substance. It remains a heat-reversible system. In configuration the sole change in the particular cases examined was a decrease in the surface of contact of the conjugates. In the second case the primary system is destroyed and a new one formed belonging to another group of colloidal mixtures. With this change an entirely new configuration appears.

All the fixing agents examined act on egg-white as formaline does upon gelatine, with the possible exception of osmic acid in minimal amounts. Therefore an irreversible change is produced, and a true insoluble phase having the typical open net structure, even when the concentration is pushed as high as 60% solids is the result.

The entire process, as we have seen, can be completely followed in the case of heat fixation, by using dilute solutions, containing only 1·0 to 1·5 grams of coagulable solids per 100 c.c.

Two stages are then clearly recognisable; in the first there is

formed by the action of temperature upon the egg-white a colloidal mixture of the silicic acid and water type, the dissolved colloidal matter undergoing a process of desolution in the change; in the second the new system so produced, following its own laws, forms irreversible molecular aggregates which may or may not link themselves together to form the open network of solid according to the concentration of the colloidal solid, the concentration and nature of the electrolytes present, and the temperature. This statement constitutes a first approximate conception of the physical events in fixation when an insoluble modification is produced.

The conclusion that two types of structure are met with in heat-soluble gels can fortunately be freed from the doubt which would always attach to it, if it depended solely upon the interpretation of appearances seen with high magnification. The transition from the gel built up of adherent droplets of solid solution—the solid-within type—to the gel built of a continuous solid frame with enclosed droplets of fluid solution, or the solid-without type, can be followed in ternary systems with lenses of a focal length long enough to eliminate diffraction errors. And, secondly, the inversion of the system is marked in the pressure experiments by a great rise in the pressure necessary to separate the conjugates, so that from pounds on the square inch it rises to hundredweights. If, however, the solid-without system is mechanically destroyed, the fluid conjugate can be separated by comparatively slight pressure. Thus the fluid conjugate is separated by a centrifugal machine if a layer of fine sand is placed above the gel. The sand particles are driven through the gel, and in their passage they cut open the walls of the fluid droplets.

For the same reason, namely the ease with which fluid can be expressed from it, we may rest absolutely certain that the figure of the true insoluble gel, the gel of silicic acid, of egg-white fixed by heat, sublimate, etc., is that of an open net. On the other hand, the detailed configuration of this net is arrived at solely by inference from an image, the dimensions of the parts of which are in some cases of the same order as the diffraction phenomena of a lens of 2 mm. focal length—that is of the lens used to produce the image.

The effect of fixing reagents upon a pre-existent net of insoluble colloid. This point was examined at some length. An insoluble gel was made from 13 % and 6·5 % of solutions of egg-white with the aid of potassium sulphocyanate. Excess salt was allowed to diffuse out into distilled water, and the final product was a firm white jelly in which

a net could be demonstrated by sections cut with the freezing microtome. Portions of this jelly were treated, just as though they were bits of tissue, with 1 % ammonium bichromate, osmic vapour, or the saturate solution of corrosive sublimate. Sections were cut from paraffin.

The results of the various experiments were the following. The pre-existent net is not markedly altered by treatment with fixing reagents. The meshes were distinctly smaller, but the figure was otherwise unchanged. The meshes were smallest in the specimens which had been treated with the vapour of osmic acid. That, however, I regarded as the effect of slight drying and consequent shrinkage of the mass.

Influence of solid particles when present in a colloidal solution before fixation. This appears to me to be a particularly important point from its practical bearing. Carmine of various grades of fineness was prepared by suspending ordinary carmine in distilled water, and separating the precipitates which fell during the third and fourth hours, and the second and third twenty-four hours. The carmine so separated was added in varying quantities to solutions of white of egg, which were then fixed with corrosive sublimate and imbedded in paraffin. Examination of sections seemed to establish the following points:—

(1) The carmine grains occupy nodal points in the net and modify within wide limits the size of the meshes and therefore also the thickness of the bars.

(2) The larger the grains and, up to certain limits, the fewer, the wider are the meshes and the thicker the bars. The grains of carmine commonly occurred in patches between which one had regions fairly free from solid particles. A study of such sections showed that when the grains were larger than the mesh in the grain-free parts of the colloid—that is to say the mesh formed in the absence of solid particles—the effect of a group of grains was to cause the mesh to widen out very much. If the grains are sufficiently numerous then each nodal point contains, or is formed, by one. If however they are too few and scattered the normal net is formed, but opens out here and there to lodge a single grain, or a group of grains on its nodal points (Fig. 2). So we may conclude that the grains modify the size of the meshes and the thickness of the bars by their number and their size.

Carmine grains correspond in their insoluble solid nature with the granule of the secretory cells of for instance the frog's pancreas and

the coarsely granular blood cells of the same animal; and not with such soluble, swellable granules as those of mucin secretory cells, or of the coarsely granular blood cells of the crayfish.

Fixation under stress. In thinking over the nature of the evidence which supports the view that the cell protoplasm has a fibrillar or reticular structure I came to the conclusion that the spindle and aster of dividing cells furnished the strongest proof. It occurred to me however that the fibrous appearance seen in cells under these conditions might be due not to a pre-existent *structure*, but to pre-existent stresses which would fashion the figure of the net, so that the latter would be, as it were, a coarse diagram of a dynamic phase of the cell history. I was not then aware that Bütschli had actually shown that a fibrillate appearance can be seen in the threads of coagulated albumen which are formed when "filtered albumen is sprinkled by means of a paint-brush into a drop of picro-sulphuric-osmic acid on a slide," and that he had come to the conclusion that "the fibrillar structure had already been produced before the coagulation by the drawing out of the fluid white of egg into threads[1]."

My own experiments differ slightly from those of Bütschli. They included the fixation of films of solution when stretched, and of a solution immediately after it had been mechanically sheared. Films of soap and egg-white of fair thickness can be made to stretch across a ring of cork. When the film was made a tiny drop of mercury was rolled on to it and the film was then fixed by alcohol in the former or sublimate in the latter case. In other cases stretched films were made by pouring a colloid solution over a drop of mercury on the slide. A fibrillar appearance radiating from the centre of application of the stress can be seen in either case after fixation. Another method is to draw a small quantity of colloidal solution along a slide with *e.g.* the point of a needle or a glass rod and then to fix it at once. In favourable cases the appearance of fibrillæ is so striking that they look as if one might isolate them by teasing.

The phenomenon is due to the well-known "after action" phenomena of colloids; which in turn is due to the fact that they are capable of retaining a shear strain. When colloidal fluids are sheared they become doubly refractive, and in this way Maxwell[2] demonstrates the existence of a shear strain in Canada balsam. The "rate of relaxation," as Maxwell called it, of balsam is however so great that the double

[1] *Protoplasm and Microscopic Foams*, p. 218.

[2] *Proc. Roy. Soc.* XXII. p. 46. 1873.

refraction does not survive this shearing motion. Klocke[1] demonstrated double refraction due to strain in a way which especially touches our subject. He stretched thin films of gelatine over tinfoil frames; then, as the films dried, they tended to contract but were unable to do so owing to the rigid frames. The films therefore became strained in drying and when dry they were found to be doubly refractive. The more viscid the colloid the more marked is the "survival" shear as one might call it. This is obvious since the phenomenon depends upon internal friction. Therefore, as Kundt[2] showed it, as too elusive to be demonstrated at all in pure fluids. Soft wax on the contrary manifests the phenomenon to an amazing extent[3]. Now we may look upon the undoubted fact that an internal strain is as it were visible after coagulation of a colloid mass in the fibrillar aspect and disposition of the fibrils in the direction of the lines of force in two ways.

We may say that shearing a colloidal mass, fluid or solid, actually does produce internal heterogeneity or, simply, structure, which is fixed by the process of coagulation. Or we may say that, though the strain does unquestionably dispose the molecules in some definite linear pattern, as is clearly proved by the phenomenon of double refraction, yet the pattern is not identical with that found in the coagulated colloid, though it certainly does condition the rearrangement of solid and liquid in such a way as to produce that pattern.

Whichever be true it is clear that neither view affords any support whatever to the hypothesis that the cell-substance is composed of two different kinds of material. The internal structure developed as a result of strain may appear in a mass previously homogeneous and has nothing in common with for instance filar and interfilar substances, or with spongioplasm and hyaloplasm, as those words are used by the histologist.

The more purely physical aspect of this last question is of interest. In the first place, if we define a fluid by an inversion of Maxwell's definition of a solid as a mass which is incapable of maintaining its shape when exposed to forces which are not equal in all directions,

[1] *Neues Jahrbuch f. Mineralogie*, II. 1881; or Lehmann's *Molecular Physics*, Vol. I. p. 533.

[2] *Ann. Phys. Chem.* XIII. p. 110. 1881.

[3] Kohlrausch, quoted by Lehmann, *loc. cit.* Speaking of the after-movements of a wax cylinder he says, "Ich kenne wenig so überraschende Vorgänge, wie diese freiwilligegen Bewegungsänderungen eines leblosen Körpers."

then it is clear that colloidal solutions depart from the definition and approach the solid state just so far as their molecules have fixed positions in the mass and are therefore capable of manifesting "survival" shear. That view of the liquid state first set forth by Poisson and developed by Maxwell which has been revived lately by Poynting offers an explanation of the phenomenon. According to it a liquid may be "in the main a solid structure inasmuch as the molecules cohere and resist strain of any kind. But the molecules have so much energy that.....the solid structure is continually breaking down and renewing itself. If we impose a shear strain on the structure, the strain will of course disappear with the structure in which it is produced[1]." Colloidal mixtures of fluid and solid depart from this fluid condition inasmuch as the strain does persist for a certain period; and this would imply an equivalent survival of the structure in which it was produced.

The principle involved in this discussion may be applied to an observation made by Engelmann. Threads of cell-substance such as for instance the fine pseudopodia of Foraminifera frequently manifest a double streaming; the direction of the stream on one side of the thread being outwards, on the other inwards. Engelmann showed that the thread under these conditions is doubly refractive and, in the light of what has been said, we may regard this as due to the shear strain imposed upon the substance at, and on each side of, the surface of separation of the two streams.

The effect of cold upon colloids. This investigation yielded results of some interest to the histologist. If a portion of a gel containing 1·5 % approximately pure gelatine be cooled while on the stage of the microscope it shows no change which can be detected by a magnification of 450 diameters until the temperature has fallen to 1° C. when exceedingly minute droplets about 0·5 μ in diameter appear in its substance. At about $-1°$ C. there is a very rapid change from this condition to a honeycomb or sponge-like arrangement[2]. The solid matter disposes itself in bars or lamellæ having a very constant thickness of about 2 μ. The meshes of the structure are rounded and have an internal diameter of 20 to 30 μ. Fluid can readily be expressed from such a sponge, and it was found to contain 0·1 % of solid matter. I was unable to satisfy myself as to the nature of

[1] *Phil. Mag.* XLII. p. 289. 1896.

[2] Temperatures were more carefully determined for 2 % agar. The sponge appears at $-5·75°$. Even ten minutes exposure to $-4·5°$ or $-·5°$ produces only slight cloudin due to formation of evenly dispersed droplets each about 0·5 μ in diameter.

this small amount of solid. At a slightly lower temperature the fluid within the spaces deposits a mass of ice crystals. The gel is transparent, or at any rate translucent, until the ice crystals appear. There are thus three stages of the solid; the ordinary gel which exists between 26° and 1°; the condition with minute droplets and the honeycomb with fluid-containing spaces 1° to −1°, and the stage with ice crystals from −1° downwards. The changes in the second stage are so rapid that I succeeded in recognising the beginning and the end only. There is therefore as it were a critical point about zero in the case of the gelatine, or about −5·5° in the case of 2 % agar when the gel assumes the structural features of an "insoluble" gel. The reverse changes differ very much in the temperatures at which they occur. The quite unimportant point, namely, the melting of the ice crystals, occurs at the same temperature as their formation, and the transparent or translucent spongy gel holding fluid is reformed. This retains its sponge-like character until a temperature of about 26° is reached in the case of gelatine, and ±75° in the case of agar, when it passes almost abruptly into the fluid state[1].

If the cooling is carried only so far as to cause the appearance of the small droplets and the temperature is then allowed to rise, the droplets, unlike the sponge structure, gradually shrink and disappear and the gel once more becomes glass-like and optically homogeneous.

If the melted gelatine is very rapidly cooled to the critical point the first stage of the solid condition is completely cut out. Thus, if a small drop of 1·5 % gelatine solution at 90° be placed on a glass plate cooled to −15° the edges at once become solid owing to the formation of a sponge the bars of which project into the central still fluid portion.

The spongy gel formed by the action of cold therefore is stable over a large range of temperature, and within this range it behaves in some respects like an insoluble gel, in that it undergoes spontaneous shrinkage with expression of fluid, and the shrinkage, the synæresis, is hastened by a rise of temperature up to a certain point. There is therefore true cold fixation. Cold causes a separation of solid and liquid; and this leads to the formation of a structure which persists during a considerable rise of temperature.

The significance of these facts may be shown by applying them to a

[1] The description in the text may be condensed into this statement. When soluble gels are cooled below a certain critical temperature the new condition imposed on them is no longer simply reversible by a small rise of temperature above the point of change. The conditions of the change therefore recall the phenomena of hysteresis.

special case. They make it clear that the expression of Kuhne's muscle plasma from muscle fibres which have been frozen is no evidence of the presence of a distinct fluid constituent in the living muscle substance.

Optical homogeneity of very thin films after fixation. An observation which at first sight appears to offer conclusive proof of the hypothesis that the living cell-substance is composed of two distinct portions was made by Schäfer on leucocytes fixed by heat. He found that the thin pseudopodial expansions differed from the rest of the cell-body in showing no net and in being resistant to stains. He therefore urges that they are composed of different material—the hyaloplasm—which has flowed out of the spongioplasmic framework of the cell-body.

The observation will not bear this interpretation since thin films of a simple colloid solution, such as white of egg, show the same resistance to stains and are apparently homogeneous after fixation by heat. These unstained homogeneous films are produced at the edge of a layer of colloid and they are commonly separated by an abrupt line from the thicker part which manifests a net and stains deeply with hæmatoxylin, or other dyes.

These edge films do not owe their characters to drying since they are seen at the junction line of white of egg and olive oil when the former is coagulated, when the colloid solution is fixed while actually flowing over the surface, and also within the colloid mass itself in thin films which are formed between contiguous air-bubbles.

They may readily be made by dipping the corner of two coverslips held in the forceps into a solution of white of egg and at once fixing by momentary immersion in a steam jet. The preparation may then be examined in water, alcohol, or, after staining, in balsam. If the fixation was quickly done the colloid solution will have penetrated only a few millimetres from the corner; it was therefore fixed while still flowing. Figure 10 is a drawing of an edge film; it shows the abrupt transition from the non-staining to the staining portions. Figure 9 shows the optically structureless film at the junction of two air-bubbles.

To my mind the phenomenon deprives Schäfer's observation of any great weight. The fact that thin pseudopodial films are clear and unstaining ceases to be conclusive evidence that they are composed of material which is permanently different from the staining mass which constitutes the cell body.

The phenomenon is probably related to the fact that when colloidal mixtures separate to form conjugates a thin layer next the surface

remains homogeneous. The depth of this layer appears to bear some definite numerical relation to the diameter of the droplets.

II. Structure produced by sub-mortem or post-mortem change in the cell.

Fixation and clotting. In the foregoing sections the process of fixation is considered much as if it was the same as clotting. This has been done because the redistribution of solid and liquid is the same in both cases. In the clotting of *e.g.* blood plasma irreversible molecular aggregates are formed by a process of desolution and the aggregates become grouped together to form a solid sponge traversing the fluid.

If now we discard the distinction between clotting and coagulation and retain only one word then the following methods of production of an insoluble gel can be recognised:—

(i) *Coagulation without concurrent chemical change.* An aqueous solution of silicic acid will set to a gel on mere lapse of time, or at once if powdered graphite be added to it. A solution of celloidine in a mixture of ether and absolute alcohol coagulates when exposed to the vapour of chloroform.

(ii) *Coagulation with concurrent chemical change.* There are three modes by which this may be brought about: (*a*) by heat, (*b*) by ferments, and (*c*) by chemical reagents. In the last class falls the process of "fixation"; and somewhere in the three classes will fall the coagulation which the whole or a portion of living matter undergoes when it dies.

Therefore, and this is the practical outcome of these considerations, a relatively solid framework may be formed in the cell-substance, either (1) by what we may call the spontaneous coagulation of the death change, or (2) by the action of reagents in fixation. If a continuous framework is to be formed then, as our experiments on simple colloids show, the process of desolution must separate a considerable proportion of insoluble matter. If only a small quantity is separated a granular deposit will result.

In the process of fixation, until proof to the contrary is forthcoming, we must recognise the following possibilities. The separation of practically the whole of the solid matter as a coarse framework by such a reagent as corrosive sublimate; or a discriminative action whereby, as Fisscher's experiments show (cp. p. 161), part may be separated as a framework, part as granules.

Structure due to the presence of, or to post-mortem changes in, secretion masses. The production of a false appearance of structure in cell-protoplasm by "secretion masses," as Flemming styled them, has been dealt with by histologists in special cases only.

Flemming writes that before one can take the appearances seen in preparations as true "vital structure" it is necessary to be very vigilant lest things which have no resemblance to such living structures are taken for them. The first case which he chooses as an instance of things which have no relation to living structure is the net seen in gland cells, for these cells hold (1) cell-substance, (2) secretion masses in "secretion vacuoles," and "it is very possible that the cell-protoplasm between the secretion vacuoles are pressed out into such thin masses (Bälkchen) that they become the same as or equivalent to the threads in cells of other kinds[1]."

Flemming however at the time he wrote could scarcely appreciate how fruitful a source of artifact these secretion masses may be: this wider knowledge follows from Langley's work upon mucous salivary glands published in 1886,—that is to say, four years after Flemming wrote.

In no structure is a more beautiful and more definite "net" observable than in the secretory cells of the alveoli of these glands, yet Langley was able to show that this net only appears as a result of a post-mortem swelling of the secretory granules which distends the cell-protoplasm.

Secretion masses, to still use Flemming's words, not only produce deformation of the cell-protoplasm by post-mortem change in themselves, but, as in the case of the net which Kupffer described in the liver cells of winter frogs, the mere presence of these masses, glycogenic in this case, implies that the whole of the cell-protoplasm is thrown into a structure or framework to hold them. In this case one can hardly speak of the secretion masses, with which in this connection one must group simple vacuoles, as sources of artifact; yet they need mention here since in many cases the net or sponge structure which writers have described as occurring *within* the cell-protoplasm is as a matter of fact something quite different, namely a frame formed of the whole of this substance, and not from a part only. A single instance may be taken to exemplify this.

Probably no cells have furnished more observations which have been

[1] *Zellsubstanz, Kern und Zelltheilung*, Leipzig, 1882, p. 60.

used to support the hypothesis of the existence of a net or sponge-work in protoplasm than the blood corpuscles of Astacus. Frommann, Heitzmann, Leydig and Griesbach may be quoted amongst others who have described the "Gerüst" in these cells. Yet it is certain that the sponge-work figured by these authors is the very obvious and beautiful sponge-work which in almost all preparations of the blood cells of Astacus is produced by the disappearance of the soluble and apparently semi-fluid oxyphil granules contained in the corpuscles during life.

There can be no doubt on this point. It is possible to watch at leisure the solution of the granules and the formation of the sponge-work, the spaces of which are readily distorted by pressure, and so to obtain appearances truthfully figured by Griesbach[1].

The most striking instance of structure in cells charged with secretion masses is furnished by the secretory cells of mucous glands.

Opinion is still divided on the question whether the very definite and delicate network so often seen in these cells includes within itself the whole of the cell-protoplasm, and is due therefore simply to the distension and compression of the protoplasm by the swelling of the secretion granules, or whether it is a true net present in the living cell and formed of only part of the cell-protoplasm. I endeavoured to come to some conclusion by a prolonged examination of the orbital glands of kittens and puppies.

The minute structure of an alveolar cell of the orbital glands. Glands from young animals were used because it was found impossible to cut very thin sections from glands of adult animals.

The material was preserved in three ways: (1) with absolute alcohol, (2) with the vapour of osmic acid, (3) with chromic acid.

Appearances after fixation with absolute alcohol. Small pieces of gland were blotted thoroughly to remove adherent blood and lymph, and then shaken quietly in a considerable bulk of absolute alcohol for about ten minutes, after which they were removed to a fresh quantity of the fixative. After 24 hours the tissue was examined and found not to be hardened; it softened readily in solutions containing less alcohol, or in water.

Study of sections cut with the free hand. These are not infrequently exceedingly thin near the edges, offering sections 2μ and less in thickness. The sections were examined in absolute alcohol, and in xylol

[1] *Pflüger's Archiv*, L. p. 473. 1891.

when unstained; they were then irrigated in succession with 90 % alcohol containing a large amount of methylene blue, with absolute alcohol, and with xylol. Tested as carefully as possible neither the xylol nor staining fluid was found to alter the disposition of the parts. Observations were made with the Zeiss apochromatic lens, aperture 1/40, focal length 2 mm.

The granules were found in different stages of preservation. In some cells distinct granules were seen, in other cells the granules had vanished, and one had irregular flakes or masses.

The granules and the flakes or masses stained an intense opaque blue with the methylene blue. The cell-protoplasm at the base of the cell coloured a vivid green. Probably in no case had the absolute alcohol prevented all swelling of the granules. Even when quite distinct they always measured 2μ in diameter; while Langley fixes the size at 1 to $1{\cdot}5\mu$.

The configuration of the cell-protoplasm was beautifully shown in these preparations. At the edge of the preparation exceedingly thin sections of cells were found from out of which the blue staining substance had fallen. One then had without doubt a honeycomb, the spaces of which did not appear to communicate with one another. (Fig. 11). The substance of the honeycomb portion of the cell was continuous with and in no sense different from the cell-protoplasm which formed the base and sides of the cell: clearly the honeycomb structure is simply the expression of the fact that the cell-protoplasm was hollowed out to hold the secretory granules: the spaces have been slightly enlarged by the partial swelling of the granules.

The configuration of the cell-protoplasm in the cells which are occupied by intact granules cannot be directly determined since the granules colour so intensely with the methylene blue as to completely overshadow any substance between them. It can be discovered however by taking advantage of the fact that there is a certain critical point in the process of inhibition of water, when the staining properties of cell-protoplasm and granules is reversed.

If a cell containing distinct granules is kept in view while the preparation is being irrigated with weaker and weaker alcohol, lightly charged with methylene blue, one witnesses the following changes. A more or less isolated cell at the edge of the section should be chosen for observation since it can expand almost without obstruction. When the

percentage of alcohol has fallen to about 60, the whole cell rapidly expands to twice its volume[1], and this is seen to be due to a swelling of the granules which, in spite of the increase in size, still remain distinct[2]. Coincident with the increase in size the granules become less deeply coloured, and their tint changes from an opaque blue to a transparent purple, while at the same time the cell-protoplasm becomes more coloured and its tint changes from green to blue. One can now see that the blue stained basal cell-protoplasm is continuous with the substance of a honeycomb in the polygonal spaces of which lie the granules. With a further fall in the percentage of alcohol the cell continues to expand in size to many times its original volume; the granules become less and less coloured until one sees only the blue stained cell-protoplasm, now expanded into exceedingly thin plates. If the percentage of alcohol has not fallen below 40, one can produce contraction of the cell by again raising the concentration of the alcohol until its volume is almost what it was at the beginning. The granules however do not recover the property of dyeing deeply with the methylene blue (Fig. 12). One can therefore stain the cell-protoplasm and so show that the spaces in the honeycomb agree in size with that of the granules before swelling occurred.

If the percentage of alcohol falls below 40, to be more accurate when it approaches 30, the distension of the cell becomes so great as to rupture the cell-protoplasm. Raising the percentage of alcohol naturally will not now restore the honeycomb.

If the percentage is kept above this destructive lower limit a cell can be swollen and contracted and swollen again an indefinite number of times without modification of the configuration at any particular stage. At no time does the material between the granules differ in staining from the cell-protoplasm round the nucleus, and it always appears to be a simple continuation of the whole of that protoplasm.

It is particularly noticeable how the granules and cell-protoplasm expand and contract together; and it is clear that the size of a cell in a preparation is no indication of the extent to which swelling may have occurred during fixation.

[1] Langley gives an account of the swelling with dilute alcohol. This *Journal*, x. p. 433. 1889.

[2] The changes in volume were computed from several linear measurements. I may therefore remind the reader that doubling the volume of a sphere means roughly an increase in a diameter from 3 to 4.

Portions of the glands were transferred to xylol after 24 hours in absolute alcohol, and imbedded in paraffin. This process, though it could not conceivably have given rise to swelling was found to have profoundly altered the physical character of the tissue, in this way, the granules retained the power of swelling though to a much less extent, while the cell-protoplasm was completely fixed and inextensible. The change is of advantage since it enables us to determine with certainty the configuration of the cell-protoplasm in those cells which contain intact granules, especially as one could use sections of 1 μ and less in thickness. These thin sections were floated with 95 % spirit on to the coverslip; and the paraffin removed by xylol. The same cell and the same alveolus were measured at various stages while the preparation was being irrigated with 80, 75, 60, 55 % and so on down to 30 % alcohol. Neither alveoli nor individual cells showed any increase in size along any axis; on the other hand measurement of the diameters of individual granules gave the following average results—in absolute 2 μ, in 50 % 2·7 μ, in 30 % 3·3 μ. In these thin sections, since the thickness is less than that of a single granule, these bodies are not restrained from swelling by the bars of cell-substance: they can expand at right angles to the plane of the section and over the bars.

Now in these thin sections from paraffin it was found that the cell-protoplasm could be stained with methylene blue while the granules remained almost uncoloured when the percentage of alcohol fell below 40. One then has a green-blue stained cell-protoplasm at the base of the cell which continues into a continuous network. Seeing that the whole cell has not changed its dimensions we may take the meshes as being unaltered in size by the irrigation. Actual measurement of meshes and of the granules before irrigation, and counting both meshes and granules proves (1) that there are the same number of meshes as granules, and (2) that the granules must incompletely fill the meshes when they are unswollen.

That the net is the section of a honeycomb is proved by this consideration. The section is less in thickness than half the average diameter of a mesh. If it were a true net composed of threads a section so thin would show broken lines joining the nodes; instead of that one always found continuous lines.

The study of these absolute fixed glands appears to me to prove that the typical net of the mucous secretory cell is the optical or actual

section of the honeycombed cell-protoplasm distended by the swelling of the secretory granules, and that it is not a net within, but a net composed of the whole of, the cell-protoplasm.

These conclusions were confirmed by the study of specially thin sections from glands hardened in chromic acid which were stained in various ways, notably with iron-hæmatoxylin.

It is difficult to raise a histological question above the region of individual judgment to the level of secure proof. In the case we are considering this fortunately can be done, by comparing the configuration of cells fixed with osmic vapour with that found in absolute or chromic fixed cells in which the granules have partly or wholly swollen. It can be clearly established that when the granules are completely preserved the configuration is totally different to the honeycomb already described; it becomes the conventional net of fixed colloids which has the granules at the nodal points; such a net as is formed when egg-white holding carmine granules is fixed.

Appearances after fixation with the vapour of osmic acid. Special precautions were taken to prevent swelling of the granules. Very small pieces of the tissue, about 2 millimetres across, were suspended in the vapour for 8, 24, or 96 hours in the dark. They were transferred either to absolute alcohol, and then to xylol, or direct to xylol, and imbedded in paraffin. Sections were floated on the coverslip with 95 % alcohol. They were examined unstained in xylol, in absolute alcohol and in lower alcohols; or after staining with methylene blue saturated in 90 % alcohol, or with iron-hæmatoxylin. The latter method was of necessity modified in order to avoid the injurious action of water. In place of the solutions recommended by Martin Heidenhain I used a saturated solution of the ammonio-ferric alum in 80 % ethylic alcohol[1], and a strong solution of Grübler's purest hæmatoxylin in the same strength of alcohol.

After 8 hours' exposure to the fixative it was found that the granules still retained the power of absorbing water; but it had almost or completely vanished after 24 hours' exposure.

The examination of unstained preparations in absolute and xylol shows that even with the small pieces of tissue which were used the granules are not preserved throughout. In by far the greater number

[1] Here and elsewhere in the paper by the word alcohol I mean ethylic alcohol, and I distinguish between solutions of this body and solutions of commercial spirit.

of the cells they were quite distinct, in a few however they had run together; in some cells the granules had "smeared" in places, four to a dozen or more having stuck together, while over the rest of the cell they were completely separate. One could therefore trace in the most complete way the change in the configuration of the cell-protoplasm induced by the smearing of the granules.

The configuration of the cell-substance when the granules were perfectly preserved was found to be that of a sponge-work of threads holding the granules on the nodal points. The form of the net varies according to whether the granules are very close set or not. In the former case the threads run simply from granule to granule, and iron-hæmatoxylin preparations of sections about 0·5 μ thick (less than one tooth) convey the impression that each granule is enclosed in a thin shell of cell-protoplasm from which bars run to the similar shell on neighbouring granules. Where the granules are less closely set the cell-protoplasm forms a net with nodal points of its own substance which stretches between the granules. A reference to the camera lucida drawings reproduced in Figs. 13, 14, and 15 will obviate further description.

The meshes of the sponge are simple fluid-holding spaces. This was proved in two ways.

(1) By microscopical examination. After 24 hours' exposure to osmic vapour the configuration was found to be unaltered by irrigation with aqueous fluids. After many failures, I succeeded on several occasions in keeping the same alveolus in the field of the microscope, with the large Abbe camera lucida always in position, for periods up to 10 days, during which time the alveolus was stained with iron-hæmatoxylin by the alcoholic method, then again by the aqueous method, and finally it was irrigated with a saturated solution of eosine and methyl eosine in water. Drawings were made at intervals, and a series from one experiment are reproduced in figures 14 *a* to 14 *d*. In other cases the alveolus was irrigated with a 20 % solution of acid fuchsine in water saturated with aniline oil, and various saturated solutions of basic dyes were tried. In short, by every method which suggested itself to me I tried to stain material in the meshes. If there be any present, then it must have these negative characters: it is invisible in fluids whose refractive index varies from that of absolute alcohol to that of Canada balsam; it cannot be stained by eosine, methyl eosine, acid fuchsine, iron-hæmatoxylin, methylene blue, and osmic acid.

(2) By direct measurement of the volume of the fluid-holding interspaces in the tissue. This was done in the following way. A number of the tiny pieces of gland were removed from the absolute alcohol in which they had been placed after fixation, and surface-dried by being rolled about on fine filter-paper. Their volume was then determined by measuring the amount of absolute alcohol they displaced. They were then again dried by being rolled on the filter-paper for as nearly as possible the same length of time, and at once compressed by screw pressure between two glass plates. The fluid which exuded was collected on dried and weighed filter-paper, which was at once enclosed in a weighed air-tight capsule, and the whole weighed. The fluid was colourless, and on evaporation left no solid residue; it was, therefore, assumed to be absolute alcohol of the specific gravity of that used to preserve the gland. The specific gravity being arrived at in this way, the volume was calculated from the weight. I was not able to collect all the expressed fluid: part remained and was visible between the small masses of gland. I was only able to collect the overflow. There was also loss at the various stages from evaporation. The volume of the quantity collected represented 46 % of the total volume of the bits before compression. Now examination of sections appeared to show conclusively, that the interalveolar clefts and the lumina taken together could not represent anything approaching 50 % of the volume of the mass; part, and probably the greater part, of the fluid, must have been expressed from the sponge-work of the alveolar cells.

The study of material which has been exposed to the fixative vapour for only eight hours affords the strongest confirmation of the account which has been given of the configuration of the cell-substance. Unstained sections examined in absolute alcohol show the granules distinctly, but it is difficult to determine the disposition of the cell-substance, owing to the diffraction halo round the granules. The disposition of the granules can, however, be exactly planned, and one has, (1) cells with distinct granules only, (2) cells with granules for the most part distinct, but here and there in heaps forming a mass having a curdled aspect, (3) cells wholly occupied by an irresolvable curdled mass. On irrigation with xylol the cell-substance becomes visible; it is now found that the separate granules are joined by threads, while the heaps of granules, and the cells in which the granules have completely run together, show the characteristic honeycomb net. Staining with

iron-hæmatoxylin and methylene blue, while still in the field of the microscope, does not alter the picture. Irrigation with lower alcohols causes the granules to swell; without, however, producing a honeycomb net in regions where the granules had been completely separate at the commencement. Figures 13 *a* and 13 *b* show the same cells as they appeared in 95 % spirit and in 30 % spirit.

It might be urged that the net structure which is characteristic of osmic vapour preparations might be produced by the granules first swelling so as to touch, and then shrinking, leaving however a thread joining the granules. Langley actually saw granules do this. This supposition is untenable for the following reasons. The granules stain a deep opaque blue with the alcoholic methylene blue, while the basal cell-protoplasm and the threads stain a true translucent green. Flooding the field with light makes the contrast in the staining reaction very obvious. Further, the threads of the network may be seen to be continuous with the basal cell-protoplasm. Lastly, if these threads are formed from granule substance, what has become of the cell-protoplasm? As we have seen, the thinnest section and the most drastic staining fails to show any substance other than granules, and the threads which link them together, or which, in more open spaces, form an independent net.

The study of this material which has been only lightly fixed by the vapour offers still further confirmation of the statement that the meshes of the sponge-work are simple fluid-holding spaces. When a preparation is irrigated with lower alcohols the granules may be seen to expand in the plane of the section, the threads of cell-protoplasm are displaced by this so that they appear as bits irregularly placed and wedged between the swollen granules. This process can be followed on the slide by irrigating with lower alcohols and then staining with methylene blue or with iron-hæmatoxylin. As one follows the process it is evident that the granules expand freely without having to displace solid matter other than the threads, and these are readily moved.

III. The interpretation of structure seen in fixed and fresh cell-substance.

The preceding pages constitute a general criticism on the inferences which have been drawn from the study of fixed and fresh cell-substance. They may be reduced to three propositions:—

(i) That a regular geometrical figure may be conferred on the cell-protoplasm by the presence in it of secretion masses and especially by post-mortem swelling of secretion masses.

(ii) That, so far as can be judged by control experiments with colloid masses of known character, the radical changes in the physical characters of the cell-substance produced by fixation are of such a nature as necessarily to produce a structure.

(iii) That the process of dying without fixation, since it appears to consist in all cases of a coagulation of some part of the cell-substance, must also produce structure not present in full life.

The study of the action of fixatives upon colloidal solution and upon soluble gels cannot be applied to the elucidation of the effect of fixation upon living cells until we know whether the living substance reacts as a soluble colloid.

If it can be shown that the configuration of the cell-protoplasm after fixation depends upon the fixative used, and upon the size and physical characters of granules which may be present, the conclusion is rendered probable.

The following tissues were examined:—pancreas, mucous coat of duodenum and stomach of frog, gut of Oniscus, red marrow and orbital gland of kittens and puppies, and peritoneal fluid of frog, and blood of the crayfish. Very small bits of tissue were taken and separated from adherent connective tissue as much as possible, since the presence of this substance makes it difficult to obtain sections of less than 1 μ in thickness. Corrosive sublimate saturated in 0·6 % sodium chloride and the vapour of 2 % osmic acid were used as fixatives. The tissue was exposed to their action for from two to four hours; then washed in 0·6 % salt solution for about 15 minutes, dehydrated by very gradually raising the percentage of alcohol, and imbedded in paraffin from cedar oil. Sections varying from $\pm$ 0·5 μ to $\pm$ 3 μ thick were used.

On the whole living cell-substance does react to fixatives just as does solid or fluid soluble colloid. The facts may be summarised as follows:—

Gland cells. When the granules neither swell nor dissolve, corrosive sublimate throws the cell-substance into an open net. The figure of the net depends upon the number and size of the granules. Figures 16 *a* and 16 *b* are from different regions of the same cell of the pancreas of a frog separated by only a short space. Figure 17 is from another cell.

The vapour of osmic acid produces a much finer structure. Where there are no granules the structure appears to be a fine honeycomb and not a net. Where granules occur the honeycomb opens out, the spaces becoming larger. Figures 18 and 19 representing the structure of the mesenteron cells of Oniscus after sublimate and osmic vapour respectively will illustrate this. The difference is partly due no doubt to the general shrinkage which accompanies fixation by osmic vapour. A rough numerical estimate of this was obtained in the following way. Two specimens of Oniscus of approximately the same size were chosen, and kept without food for five days. The intestine of the one was exposed to the vapour of 2% osmic acid for two hours, that of the other was placed in the sublimate solution for the same period. They were then imbedded in paraffin by the same process and sections were cut as nearly as possible at right angles to the length of the gut.

The long and short axes of a number of nuclei in each gut were measured and the mean of the measurements were taken as the diameter of a sphere, the volume of which was calculated. It was found that the ratio of the mean volumes of a nucleus—roughly estimated in this way—in the osmic vapour preparation, to the mean volume in the sublimate preparation was 7 : 11. To put these figures in another way, if the percentage of solid in the sublimate fixed cells were 10, then it would be about 16 in the osmic vapour fixed cells.

In osmic vapour preparations of the pancreas of the frog an interesting point was noticed which showed the effect on the configuration of the cell-protoplasm of the rapidity of fixation and of shrinkage. In cells quite at the surface of a bit of tissue the spherical discontinuities in the cell-protoplasm where it was fairly free from granules were so excessively minute as to be perceptible only in sections not thicker than $\pm 0{\cdot}6\,\mu$ which were deeply stained with iron-hæmatoxylin. Passing more toward the centre of the mass of tissue one found the discontinuities in the cell-substance larger and more obvious. This effect of the rapidity of fixation demands for its elucidation a knowledge of the volume of the cells in the various conditions. This I do not possess. The facts however may fairly be set beside the account which Mikosch gives of the changes in the configuration of living cell-substance which occur as it is dying[1]. He observed the epidermic cells of various plants—chiefly *Sedum telephium.* The tissue was mounted

[1] *Verhand. d. Gesell. deutsch. Naturforsch. u. Ärzte*, Th. II. p. 179. 1895.

in water or sugar solution and when first viewed the cell-substance appeared to consist of a completely homogeneous matrix which imbedded small refractive granules commonly in rows like a string of pearls; some however were grouped in pairs, others separate. After 20 to 30 minutes minute vacuoles not more than 1μ in diameter appeared in the cell-substance. They gradually increased in size so that a net appearance was produced with rounded and polygonal meshes. The granules before mentioned have their arrangement destroyed by this change; they now lie at the nodal points of the net, and the appearance is similar to that usually figured by Bütschli. The vacuoles continue to increase in size, and they burst into one another until finally a structureless mass is produced which encloses still persisting vacuoles.

The same conclusion, namely, that the configuration of the cell-protoplasm is determined by the fixative is most clearly shown in the case of the secretory cells of the orbital gland. The facts have already been described and nothing need be added here.

In the case of the giant cells of red marrow the configuration of this cell-protoplasm after fixation with corrosive sublimate is such as to show in sections a net, the average diagonal of a mesh being about 0·6 to 0·7 μ. After fixation with osmic vapour the average measurements are at least one-fifth less.

Special mention might be made here of a criticism based upon the structure of oxyphil wandering cells after fixation. On the ground that the granules in these cells form the nodal points of the network Gulland[1] claimed that they could not be of the nature of secretory granules, as had been urged by Hankin, Kanthack, and the writer. If these granules really are secretory granules they would according to Gulland occupy the position of paraplastic matter—namely the meshes of the net. The nature of this criticism serves to bring into prominence the shifting artificial nature of the structure in fixed cells, for, as we have seen, the secretory granules of the alveolar cells of the frog's pancreas form the nodal points of the network, while the secretory granules of the orbital gland lie sometimes on the net, sometimes in the meshes, according to the nature of the fixative.

Effect of the thickness of the section upon the image. The very great difference which exists between the image offered by sections which do

[1] This *Journal*, XIX. p. 385. 1896.

not vary very much in thickness will scarcely be realised except by the aid of actual observation.

In a section approximately 1 μ in thickness through a gut of Oniscus which has been fixed with sublimate the cell-substance shows as an open structure like the skeleton of a dead leaf. In sections say 4 μ and upwards in thickness the appearance presented is that of a coarsely punctate material.

The point was studied in detail in cells of the red marrow fixed with sublimate. The lenses used were the same Zeiss apochromatic with the ocular 18 (*i.e.* × 2250 diameters). Sections of three grades of thickness were compared; the thinnest were about the thickness of the visual field, the next were between two and three visual fields in thickness[1]. The thickest sections were too thick for this method of estimation, but they were roughly twice as thick as the medium sections. Now, in the thinnest sections one has a clear open mesh such as is shown in Fig. 20. As the sections increase in thickness one has more and more decidedly the impression that the net at any particular focal plane lies imbedded in a faint continuous grey ground substance. This impression is due to the haze from the focal planes above and below the one actually in focus. Figures 20 *a*, *b* and *c* are from a cell in a section of medium thickness. The section of the cell was a little more than two visual fields in depth. Figure 20 *a* is a free-hand drawing of the upper visual field. Figure 20 *b* a similar drawing of the lower field. Figure 20 *c* is a drawing made with the camera lucida, the focal level being that of the upper visual field.

In addition to the confusion in the image which results from the fog so to speak of material out of focus, there is a possible source of error in what might be called false differentiation by double staining. This may be explained by citing an instance. In many or most cases the bars of a net differ in thickness in different parts of the cell. If a section is stained with iron-hæmatoxylin and the process of washing out be used, the dye is first removed from these finer structures. On double staining with say eosine the fine bars from which the hæmatoxylin has been removed dye red and one then has a net black in some places, red in others. The whole process can be followed in the field of the microscope.

[1] Absolute measurements in μ represent the relation less accurately. It is easy to convince oneself, both by reasoning and practice, that the possible error in absolute measurement is fully equal to the depth of a visual field.

The foregoing paragraphs may be summarised as follows:—The study of the behaviour of certain gland cells and of the cells of red marrow, of frog's lymph and of the intestine of the wood-louse, leads me to conclude that the cell-protoplasm reacts to corrosive sublimate and osmic vapour in the same way as does a soluble colloid to a reagent which converts it into an insoluble colloid. I hold therefore that there is no evidence that the structure discoverable in the cell-substance of these cells after fixation has any counterpart in the cell when living. A large part of it is an artifact. The profound difference in the minute structure of a secretory cell of a mucous gland according to the reagent which is used to fix it would, it seems to me, almost suffice to substantiate this statement in the absence of other evidence. The framework which is visible in fixed cells, contains within itself all the solids of the cell; it is produced by the action of the fixing reagent in converting the $\pm 10\%$ of solids in the living cell-substance into an insoluble state. The meshes of the framework therefore are mere interstices occupied by alcohol, xylol, or balsam as the case may be. This conclusion is borne out not only by microscopical analysis and pressure experiments, but also by argument from the physical characters of colloidal matter in the soluble and insoluble states.

Though it is impossible to deal with questions of such magnitude in anything other than a suggestive manner it would be unfair to omit pointing out the lengths to which criticism of this kind carries one. I have confined myself in this work to a consideration of those views which ascribe definite coarse structures to the cell-protoplasm: but the dead or fixed cell body also holds other structures—the nucleus and the attraction sphere, each of which represents something which is present in the living cell. The same difficulty exists in determining the structure of these bodies during life as exists in the case of the general cell-protoplasm—it is the difficulty of deciding to what extent structure demonstrable in the fresh or fixed state is the product of the chemical and physical changes which constitute the death change, or which may be due to the action of fixatives.

In discussing the view of Berthold and Fr. Schwarz according to which the reticular structure of fixed cell-protoplasm is an artifact Bütschli appeals to the structure which various workers have described in "living protoplasm." These observations satisfy him that criticism such as was advanced by Berthold and Schwarz and as appears in the pages of this paper is false and "requires no further

refutation." It is valueless in face of the fact that net or fibrillar structures "are frequently to be observed quite plainly in the living condition and therefore cannot be any artificially produced appearances of precipitation or coagulation."

To this point of view there are two fundamental objections. The first may stand in the form of a question. As Bütschli has himself contributed to show, fixatives do produce structures in colloids; is not therefore the statement that the structure seen in the fixed cell agrees with that seen in the unfixed cell singularly suspicious? What has become of the structure which must be produced by those complete redistributions of solid and liquid and those chemical changes which are the very essence of the process called fixation? In the second place an examination of the original memoirs convinces me that it is very doubtful whether the structure in question has been observed in actually normal living cells. The discussion of this point, together with an account of some attempts to repeat the more noteworthy observations upon cells regarded as living will form the subject-matter of the second part of this paper.

SUMMARY.

(1) A study of the action of reagents upon colloidal matter shows that when an insoluble modification is formed there is a separation of solid particles which are large molecular aggregates, and that these become linked together to form a comparatively coarse solid framework having the form of an open net which holds fluid in its meshes.

(2) In some cases however the reagent is partially miscible with the colloidal mixture. In this case the latter is modified in degree but remains the same in kind. The action of corrosive sublimate upon gelatine is an instance.

(3) The general statement is however possible that reagents which have any action at all confer a structure upon the colloidal matter which differs in most cases in kind, in some cases in degree, from the initial structure. Hence it is inferred that the structure seen in cells after fixation is due to an unknown extent to the action of the fixing reagents.

(4) The structure of dead matter which was once living may also be referred to the coagulation (clotting) phenomena of death, as well as to post-mortem change. These points were specially examined in the case of the secretory cells of a mucous gland.

(5) Incidentally certain investigations which are still in progress as to the phenomena of the colloid state are touched upon. The existence of two classes of colloidal mixtures is noted and the chief features of each are described.

(6) The mechanism of coagulation, and of setting, receives special discussion.

LIST OF FIGURES.

Egg-white. Sections 0·6 to 1·0 μ thick cut from paraffin; stained with iron-hæmatoxylin. Magnified 1500 diameters; camera lucida.

Fig. 1. Solids per 100 c.c. 13 grs. Fixative sublimate.
Fig. 2. Solids per 100 c.c. 30 grs. Fixative sublimate. *a. a.* Carmine grains.
Fig. 3. Solids per 100 c.c. 60 grs. Fixative sublimate.
Fig. 4. Solids per 100 c.c. 13 grs. Fixative potassium sulphocyanate.

Gelatine. Film preparations fixed with sublimate. Mag. 1500 diams.; camera lucida.

Fig. 5. Solids per 100 c.c. 4 grs.
Fig. 6. Solids per 100 c.c. 10 grs.
Fig. 7. Solids per 100 c.c. 25 grs.
Fig. 8. Solids per 100 c.c. 50 grs.

Fig. 9. Egg-albumen. Solids 13 grs. per 100 c.c. Film fixed by steam while flowing between two coverslips—to show abrupt transition from net region to optically homogeneous film. *a*, *a'*, interior of two contiguous air-bubbles. Mag. 450 diams.; camera lucida.

Fig. 10. Gelatine droplet fixed while flowing over coverslip with sublimate. Stained with iron-hæmatoxylin. Note unstaining optically homogeneous edge film. Mag. 1000 diams.; camera lucida.

Orbital gland. Kitten.

Fig. 11. Fixative absolute alcohol 20 hours. Freehand sections stained with methylene blue in 90 % alcohol and examined in xylol. Framework a brilliant green, shrunken remains of mucous granules a dull opaque blue. Mag. 1500 diams.; camera lucida.

Fig. 12. (*a*) Net in 50 % spirit; intact granules swollen. *Not* camera lucida.
(*b*) Same net, after dehydration in xylol; methylene blue.

Fig. 13. Fixative osmic vapour 8 hours. Section 2 teeth ($\pm$ 1·4 μ) cut in paraffin. Mag. 1000 diams.; camera lucida. (*a*) Appearance after removal of paraffin and mounting in 95 % spirit. (*b*) After irrigation with lower strengths of spirit down to 30 %; drawn in 30 %. Section unstained.

Fig. 14. Fixative osmic vapour 24 hours. Mag. 1000 diams.; camera lucida. Section 1 tooth ($\pm$ 0·7 μ). Same portion of the alveolus was drawn in each case. (*a*) section unstained mounted in absolute alcohol after removal of paraffin. Stained with iron-hæmatoxylin while on stage of microscope, brought back into xylol, drawing (*b*) made. Staining light. Restained with iron-hæmatoxylin—ferric alum 2 % 13 hours; strong hæmatoxylin 24 hours; staining very intense. Brought into absolute, drawing (*c*) made; irrigated with xylol and drawing (*d*) made. Illumination moderate, the condenser being achromatic. The change in the image due to the refractive index of the mounting medium is noteworthy. In (*c*) resolution was difficult owing to the intensity of the stain.

Fig. 15. Orbital gland. Puppy. Section 2 teeth ($\pm$ 1·4 μ). Fixative osmic vapour 24 hours. Stained with methylene blue saturated in 80 % alcohol. Mag. 1000 diams. Drawn when mounted in absolute; illumination of field varied, but mostly bright; very careful study with camera lucida of part of a cell.

Fig. 16. Pancreas. Frog. Fixative sublimate. Section 1 tooth (0·7 μ). (*a*) and (*b*) were in the same cell, (*a*) being almost continuous with (*b*). Mag. 2250 diams. Probably not made with camera lucida.

Fig. 17. Pancreas. Frog. Fixative sublimate. Section 1 tooth ($\pm$ 0·7 μ). Drawing was not made with the camera lucida. Mag. 2250 diams.

Gut of Oniscus. Animals starved for 6 days.

Fig. 18. Fixative sublimate 2 hours; no exposure to water; dehydrated in alcohol with a trace of iodine. Section 2 teeth (1·4 μ). Stain, iron-hæmatoxylin. Camera lucida.

Fig. 19. Fixative osmic vapour 2 hours. Section 1 tooth (0·7 μ). Stain, iron-hæmatoxylin. Camera lucida.

Fig. 20. Oxyphil cell of red marrow. Fixative sublimate. Section between 2 and 3 focal planes thick. Illumination moderate. Mag. 2250 diams.

(*a*) freehand drawing of upper focal plane,
(*b*) freehand drawing of next deeper plane,
(*c*) combining these two planes and adding a third.


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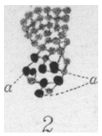

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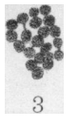
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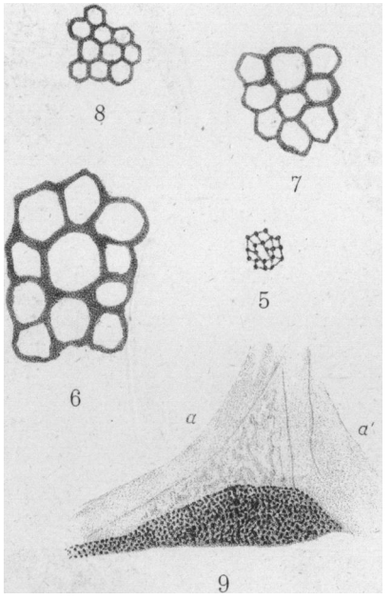

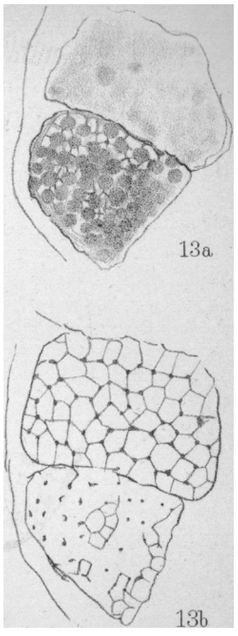

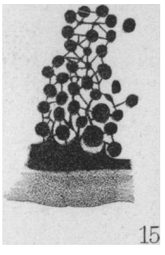
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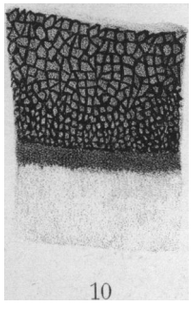
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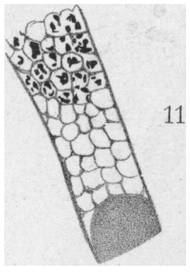
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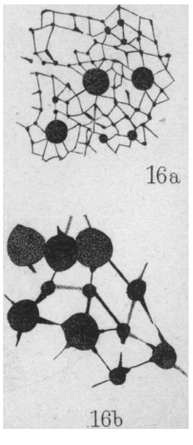


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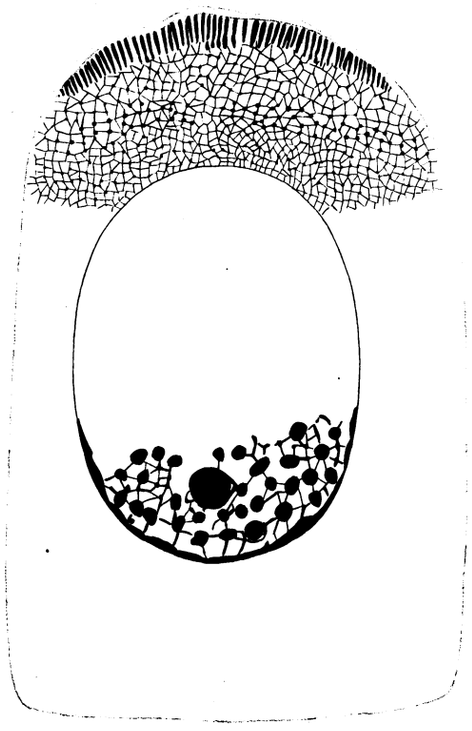

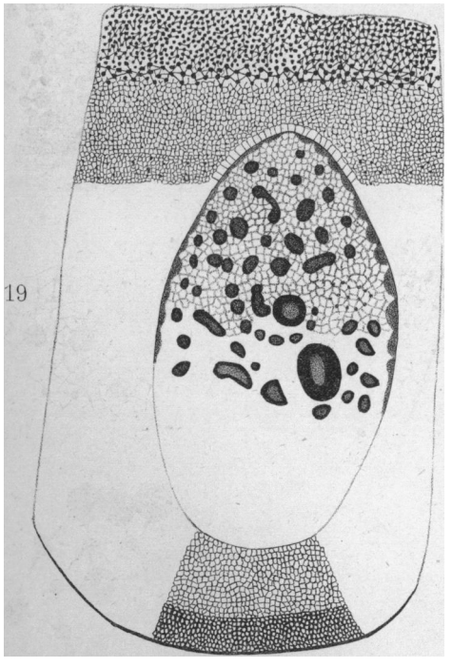

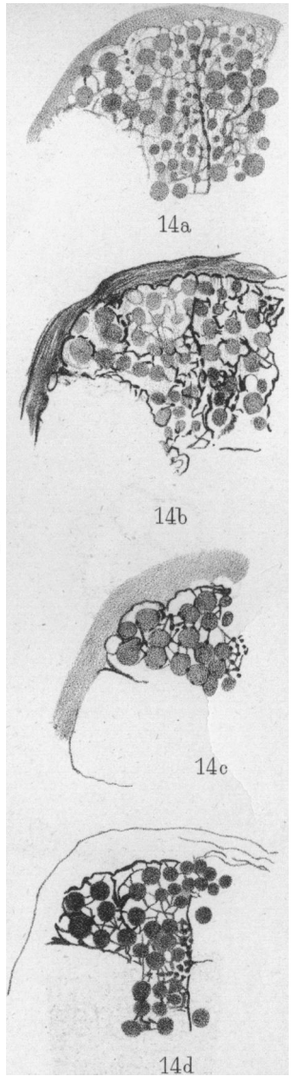

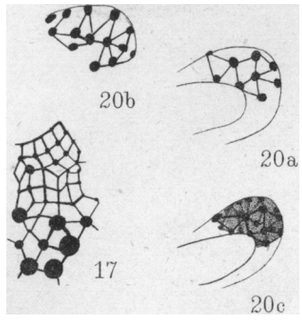

E. Wilson. lith, Cambridge.