XXXVIII. STRUCTURES IN ELASTIC GELS CAUSED BY THE FORMATION OF SEMIPERMEABLE MEMBRANES.

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WHEN a reaction is produced in a gel, the latter may undergo slight changes which, however, are not striking or even obvious, and have received little attention compared with that devoted to the characteristics of insoluble precipitates obtained in the gel. These changes are, generally speaking, alterations in the water content of the gel, such as dehydration by contact with very concentrated salt solutions, *e.g.* the 20 or 25 % silver nitrate solution used in the classical Liesegang experiment; or increased hydration caused by the diffusion of acid or of one of the strongly lyotropic salts such as iodide or thiocyanate. If the experiments are carried out in test-tubes, these changes show themselves most readily in alterations of the gel meniscus.

Much more complicated conditions arise, and the gel itself develops a complicated structure, when the reaction leads to the formation of a semi-permeable membrane. The most convenient and striking instance is the formation of copper ferrocyanide in gelatin gel, and the most suitable arrangement is as follows: clean test-tubes are filled to about half their height with a gelatin sol containing 10 g. of air dry gelatin in 100 cc. of a 2 % solution of crystallised potassium ferrocyanide ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$). After setting completely—which is somewhat retarded by the ferrocyanide—the gel is covered with a 5 % solution of crystallised cupric chloride or cupric sulphate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ or $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ respectively), or, as will be explained below, with a mixed solution of cupric and sodium sulphate. The molar concentrations are accordingly $M/21 \text{ K}_4\text{Fe}(\text{CN})_6$ in the gel, and $M/3.4 \text{ CuCl}_2$ or $M/5 \text{ CuSO}_4$ in aqueous solution. The molar concentration of the latter is thus considerably greater than the molar concentration of the salt in the gel, the necessary condition when a reaction producing an insoluble compound is to proceed into the gel.

A few minutes after putting on the copper salt solution a pale brown and perfectly transparent membrane of copper ferrocyanide can be noticed. The next change to be noticed is a gradual sinking of the surface of the gel. In the first stages there is no great alteration in the curvature of the meniscus, but it appears to travel down bodily, leaving a thin film of gel adhering to the glass. The constant formation or exposure of new surface entails fresh

formation of copper ferrocyanide, and the layer of gel on the glass as well as the meniscus turns dark brown and opaque. If the preparation is observed superficially only and at long intervals, this shifting of the meniscus and the formation of a dark brown zone on the glass seem to be all that happens. Careful observation however shows the process to be discontinuous: the curvature of the meniscus increases and finally the membrane composing it *bursts* somewhere near the centre, the copper salt solution penetrates through the crack and a fresh membrane is formed.

What happens can be readily deduced *a priori* from known properties of the semipermeable membrane and of elastic gels. As soon as the membrane is formed, water flows through it from ferrocyanide solution in the gel to the strongly hypertonic copper salt solution above it: in other words, the gel "dries." In doing so it behaves exactly as if drying proceeded in the usual way by evaporation into the atmosphere, *i.e.* the meniscus gradually sinks and becomes more and more curved. The surface film now consists of very concentrated gel and—provided the gelatin adheres well to the glass—is in tension. At the same time, owing to the withdrawal of water and the low diffusion velocity of the potassium ferrocyanide, with a molecular weight of 368.5, the concentration of the latter in the zone immediately below the membrane increases markedly; this can easily be seen by the colour, which is a clear yellow, although the rest of the gel, with the initial concentration of 2 %, is barely coloured. The ferrocyanide has, like the other cyanides, a marked softening effect on gelatin, which shows itself in concentrations of about 10 %, so that the layer below the tough surface film is considerably softer than the body of the gel. The surface film accordingly is torn off when the tension has increased sufficiently, and is at the same time ruptured by the atmospheric pressure, the copper salt solution penetrating under it. The whole process then repeats itself, and this continues until the copper salt solution, which is continually diluted, becomes approximately isotonic with the potassium ferrocyanide solution in the gel.

If the gelatin does not adhere to the glass wall sufficiently, it begins to contract below the reaction zone and copper salt solution flows into the space thus formed, covering the exposed gelatin surface with a semipermeable membrane. This leads to further shrinkage and finally a thin cylinder of very tough gel only may be left.

The analysis of the process, set forth above, was confirmed by a detailed examination of a large number of preparations. In the first instance the test-tubes were cut through about 1 cm. below the bottom of the reaction zone. Immediately the cylinder of gel is cut across, it contracts sharply and develops a deep constriction below the last membrane. The dehydrated gel containing the precipitate of copper ferrocyanide no longer adheres to the glass, and the reaction zone can, with a little care, be removed intact from the tube and cut through longitudinally. Two halves of such preparations, made in large boiling tubes 35 mm. in diameter, are shown in Fig. 1 (Plate IV).

An intact test-tube, in which the reaction has proceeded for some days, is illustrated in Fig. 2. The rupture in the last membrane but one is clearly visible, and the membrane in course of formation, which is still almost transparent, can be seen faintly. Fig. 3 shows a preparation in which the gel has detached itself from the glass and has become covered completely with copper ferrocyanide; the consequent shrinkage is very marked.

If the reaction proceeds throughout the gel, without the latter detaching itself, the final aspect of the preparation is extremely remarkable. It then consists of a thin tube of tough gel with a large number of septa, convex towards the lower end of the cylinder and always ruptured somewhere near the centre. A cross section through a specimen of this description is shown in Fig. 4. It may be mentioned that this preparation was obtained by using a mixed solution of copper and sodium sulphate, to which reference has been made, instead of copper sulphate alone. The function of the copper salt is a twofold one: it provides material for the formation of the semipermeable membrane, and it maintains the osmotic pressure so that dehydration of the gel takes place. The latter function can of course be performed equally well by a salt which does not enter into the reaction, and sodium sulphate has the advantage of not tanning the gelatin to the same extent as copper and other heavy metal sulphates. It is the latter action which seems to lead, or to contribute largely, to the detaching of the gel from the glass, which makes it difficult to obtain specimens showing more than two or three septa with copper salt alone. Incidentally the condition of the glass surface is of considerable importance: test-tubes cleaned carefully with bichromate-sulphuric acid mixture etc. consistently gave bad results, while new test-tubes simply dusted with a dry cloth gave the best.

If the explanation of the formation of septa, which has been given above, is correct, it follows that they should be formed in the course of any reaction which satisfies the following three conditions: (1) a semipermeable membrane is produced; (2) the solution above the gel is, and remains, hypertonic, and (3) the gel contains a lyotropic agent which, in moderately high concentration, reduces its modulus and tensile strength. Only one such combination—apart from the copper ferrocyanide reaction—readily suggests itself: gelatin gel containing potassium iodide as lyotropic agent, and on it a concentrated sugar solution containing tannin. The general results were the same as with copper ferrocyanide: depression of the meniscus, followed by the detachment and rupture of membranes. The latter were very irregular, probably because one set only of concentrations, chosen at random, was tried; a detailed investigation appeared unnecessary in view of the somewhat artificial nature of the arrangement.

The results described show that the possibilities of periodic structures in gels, as the result of continuous and uncontrolled diffusion, are by no means exhausted by the normal Liesegang phenomenon. In the latter the gel, while of course influencing the character of the results very considerably, primarily

serves as the support for periodic deposits of insoluble reaction product, and does not undergo any profound changes. If a semipermeable membrane is formed by the reaction, the gel itself becomes segmented and the final structure is one which it would be extremely difficult to explain *a posteriori*—quite as difficult as it was to explain other periodic structures before the study of the Liesegang rings provided a clue. While the author does not know any natural structure resembling those described and while he is, generally speaking, by no means inclined to overrate the cogency of the conclusions drawn from artificial imitations of natural forms, he ventures to think that the highly complicated consequences which follow the formation of a semipermeable membrane in a suitable elastic gel may have some biological or histological interest.

Fig. 2

Fig. 3

Fig. 1

Fig 4