XXXV. A SERIES OF ABNORMAL LIESEGANG STRATIFICATIONS.

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(With Plate VI.)

THE experiments described in the present paper represent preliminary work for an investigation in which it was necessary to produce Liesegang stratifications in gelatin specimens having volumes up to 300 cc. and completely submerged in the aqueous solution of the one component. It was therefore desirable to employ a material less expensive than silver nitrate and, for reasons apart from the cost, to produce in the gelatin a precipitate more closely corresponding to some of the mineral constituents of organic structures. I accordingly determined to study the deposition of calcium phosphate in gelatin gel.

The reaction between alkaline phosphates and calcium nitrate has been used by Stephane Leduc [1911]. He used Liesegang's procedure, which consists in placing a drop of the solution of one reaction component on a film of gelatin gel containing the other, and obtained stratifications of such fineness that they acted as a diffraction grating. It has been shown conclusively by Liesegang that this effect is due to wrinkling of the surface of the gel [1913]. This particular effect would have been useless for my purpose, which was to produce stratifications extending through a considerable depth of gel, and I accordingly studied the reaction in test tubes. This procedure is now well known: a test tube is partly filled with the sol containing one of the components in suitable concentration, the sol is allowed to set completely, and the aqueous solution of the other component—which must, in all cases, have a molar concentration greatly in excess of that in the gel—poured on.

After some preliminary experiments, which it is not necessary to describe in detail, it was found that the best results were obtained with gels containing 10 g. of gelatin in 100 cc. of trisodium phosphate solution, the concentration of the latter being 3, 2.5 or 2 % of the crystallised salt ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, *i.e.* respectively 7.87, 6.57 and 5.26 millimoles of Na_3PO_4), on which were placed solutions of calcium chloride, calcium nitrate or mixtures of calcium chloride and sodium chloride. The use of such mixtures, one constituent of which takes no part in the reaction, is a well known device for reducing the concentration of the reacting constituent, while keeping the total molar concentration—which settles the rate of diffusion into the gel—sufficiently high.

In preparing the gelatin sol a somewhat unusual procedure was adopted, except in a few instances, which will be mentioned specially. The usual course—dissolving the salt in the sol—proved objectionable, as all five brands of gelatin used in the investigation gave a, more or less copious, precipitate with trisodium phosphate. If the salt, as is usually done, was added to the sol, the protective effect of the latter rendered the precipitate so fine that the greater portion passed through the Chardin paper usually employed for filtering gelatin. The gelatin leaf was therefore allowed to swell in the phosphate solution itself: in this way whatever precipitate is produced forms in the gelatin gel, which renders it coarser even than it would be in aqueous solution. After 24 hours' swelling the gelatin was dispersed by heating to about 90° and then filtered on a hot water funnel, at about the same temperature, through Chardin paper. Treated in this way all five gelatins gave filtrates as clear as those obtained from the same gelatins without the addition of phosphate. The filtered sol, while still warm, was poured into the test-tubes and allowed to set for about 24 hours before the aqueous solutions were poured on. In some instances, which will be specially referred to, the sol was allowed to set, and the resulting gel re-melted for filling into test-tubes.

The times allowed for swelling, dispersion and gelation in the test-tubes were strictly adhered to throughout, unless a departure from the standard procedure is expressly mentioned; the time required for filtering different brands, which varied considerably, was unfortunately beyond control. As the phosphate solution is markedly alkaline, the difference in the times during which the sols were exposed to the filtering temperature of about 90° (this time varying from 30 to 90 minutes for 200 cc. of different brands) and the consequent difference in hydrolysis may, in part, account for the astonishing divergence in the results obtained with different brands in conditions otherwise alike. It may be well to add that stratifications are obtained only with trisodium phosphate, and not with either Na_2HPO_4 or NaH_2PO_4 .

Five brands of gelatin were investigated: (1) a pre-war German table gelatin of unknown origin and moderate "hardness," (2) an English calf gelatin, (3) and (4) two different brands of English gelatin of the same maker, and (5) a French photographic gelatin, the last four brands being all markedly hard. A selection of the most striking results is illustrated in Pl. VI, figs. 1 to 12.

Figs. 1, 2 and 3 show stratifications of the normal type, but of unusual perfection as regards both the regularity of the strata and the entire absence of precipitate in the clear spaces between them. The number of strata is unusually large in No. 3. The preparations were obtained as follows: No. 1, gelatin (2) with 3 % of trisodium phosphate, the aqueous solution consisting of equal parts of 10 % solutions of calcium chloride and sodium chloride; No. 2, gelatin (1) with 2 % of trisodium phosphate, the aqueous solution consisting of four parts of 10 % calcium chloride and one part of 10 % sodium chloride solution; No. 3, gelatin (1) with 2 % of trisodium phosphate, the aqueous solution containing 10 % of crystallised calcium nitrate.

Anomalous features first show themselves in No. 4. The strata are extremely numerous and regular, but are curved, the *upper* side being convex. This is quite unusual: if strata are curved at all, they are concentric with the meniscus of the gelatin gel, *i.e.* the concave side is turned upwards. In addition, many of the strata show a small opening about the centre. This specimen was obtained with gelatin (1), containing 3 % of trisodium phosphate, and an aqueous solution of 20 % of crystallised calcium nitrate.

A different anomaly is exhibited by No. 5, gelatin (4), with 3 % trisodium phosphate and a 20 % solution of calcium nitrate. Below the strata there appear rings of precipitate which are confined to the glass surface. As this brand of gelatin exhibited the same tendency with other concentrations of phosphate and other aqueous solutions, its further use was abandoned.

No. 6 is a very remarkable specimen. The gelatin was (2), with 2 % of trisodium phosphate, while the aqueous solution was a mixture of two parts of 10 % CaCl_2 and three parts of 10 % NaCl solution. The last four strata alternate with layers of macroscopic aggregates, and the distances, especially between the last two strata, are remarkably great. This alternation between zones containing a large number of small, and a small number of large, particles, has been observed before on a microscopic scale [Hatschek, 1911], but never in the striking fashion exhibited by the present specimen.

Even more striking anomalies are, however, exhibited by Nos. 7 to 12. Some of these appear to have been caused purely by slight (intentional) variations in the procedure adopted in soaking the gelatin etc. Thus in No. 7, the gelatin, phosphate concentration and the aqueous solution were exactly the same as used for No. 3. The gelatin was, however, soaked in water for 30 minutes only, dispersed by warming and the trisodium phosphate added to the sol, which was then filtered. The strata, although spaced normally, show a very marked convexity upwards, and a tendency to the formation of central stems. Similar convex strata are shown by No. 8, but these are arranged quite regularly in pairs. In this case the gelatin and the phosphate concentration were the same as in No. 1, but an aqueous solution of 20 % $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was used. Nos. 9, 10 and 11 show an even more striking feature not observed hitherto: the formation of wide and narrow bands of precipitate, which alternate regularly in No. 9 and—apparently—quite erratically in Nos. 10 and 11. In these three cases the gelatin was soaked for only 45 minutes: Nos. 9 and 11 were gelatin (2) with 2 % of trisodium phosphate, the aqueous solution being respectively 10 % $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 7.5 % CaCl_2 . No. 10 contained 2.5 % of trisodium phosphate, the brand of gelatin and the aqueous solution being the same as used in No. 9. The gel used for No. 9 had been re-melted about 24 hours after filtration.

No. 12 finally shows the result obtained in a gelatin gel *hardened by formaldehyde before* the solution of the calcium salt was allowed to diffuse into it. The gel was gelatin (1) with 2 % trisodium phosphate, which was hardened by allowing a solution containing 1 % formaldehyde and 2 %

trisodium phosphate to diffuse into it for about five days. The addition of phosphate to the formaldehyde solution is of course necessary to prevent the salt from diffusing out of the gel. After five days the formaldehyde solution was poured off and was replaced by a 10 % solution of CaCl_2 . The curious pseudo-helical arrangement has never, to my knowledge, been observed before in test-tube experiments: it recalls, however, the spirals obtained by Liesegang [1914], with the silver chromate reaction carried out in his usual manner on plates, in gelatin gels of suitable acid and gelatose content.

In the present unsatisfactory state of our knowledge regarding the normal phenomenon it is impossible even to attempt an explanation of the extremely complicated structures just described. Other reactions, such as Liesegang's original example, are also sufficiently sensitive to small variations in the brand of gelatin, its acid and gelatose content, in the concentration of the reacting solutions and in the procedure adopted. The resulting stratifications differ, however, generally speaking, in number, in the width of the chromate bands and of the spaces separating them and in the amount of diffuse precipitate found in the latter: such anomalies as bands distinctly grouped in pairs, or alternately wide and narrow, have never been described. While the results therefore enormously increase the difficulty—sufficiently great even in view of the material published hitherto—of propounding a general theory of the phenomenon, they may also enhance its importance to the biologist and geologist by showing that structures of a much more complicated nature than any hitherto obtained in such experiments may be the result of simple diffusion into a gel.

REFERENCES.

- Hatschek (1911). *J. Soc. Chem. Ind.*, No. 5, 30.
Stephane Leduc (1911). *Theorie physico-chimique de la vie*, Fig. 16.
Liesegang (1913). *Kolloid. Zeitsch.*, 12, 181.
—— (1914). *Zeitsch. physikal. Chem.*, 88, 1.