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by

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I wish to express my appreciation to Dr. E. R. Riegel for all his kind attention and assistance during the preparation of this thesis.

A Study of the Liesegang Phenomenon and of the Zone Pattern Formation in Solid Gelatin Gels.

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Section I

This investigation is an extension of the study on the Liesegang phenomenon or periodic stratification in solid gelatin gels and on the zone pattern formation first reported by Riegel and Widgoff (1). It was not the purpose of this investigation to form new combinations of salts which might result in periodic precipitations although some new combinations were formed incidently in the course of the work, but to take substances which were known to form this stratification and study this formation more in detail and under varying conditions so as to have a more complete knowledge and understanding of this occurrence.

The contributions toward this subject since Liesegang first studied this occurrence in 1896 (2) are too numerous to be reviewed here. The theories endeavoring to account for this phenomenon are also very numerous in fact their very number and variety indicates that no one of them is satisfactory in every respect. Also, it means that this occurrence is not an easy one to account for.

Hatschek says in the Second Report on Colloid Chemistry: British Association Committee, "The experimental reproduction and elucidation of natural periodic structure should for a long time to come be one of the most fruitful fields for applied colloid chemistry."

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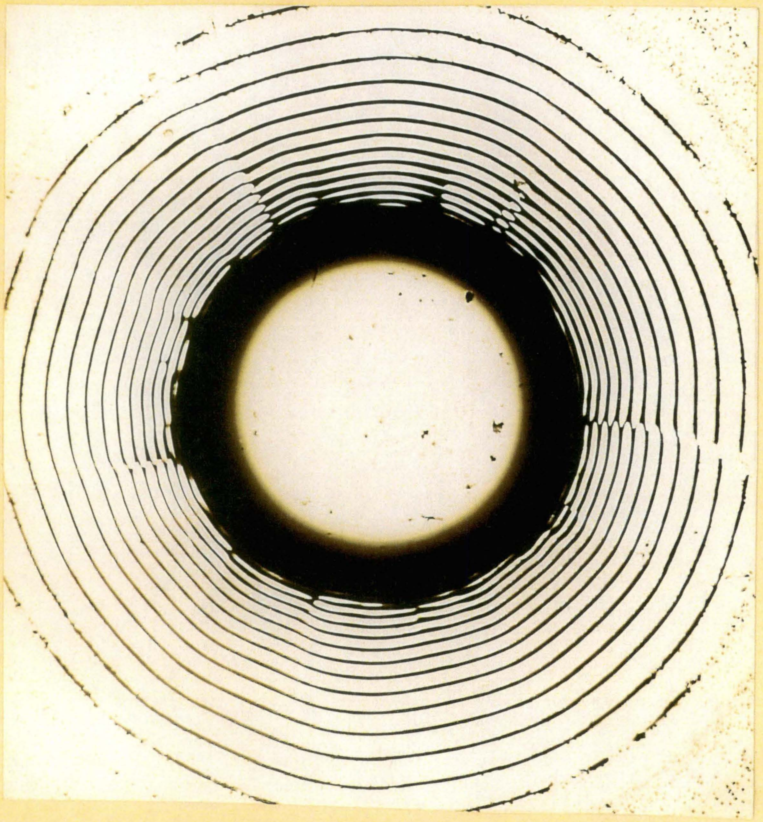
(1) Riegel and Widgoff, J.Phys.Chem.,29, 872, 1925.
(2) Liesegang, Photo.Arch. 231, (1896) "Chemisch Reaktionen in Gallerton," Dusseldorf, 1898.

What is meant by periodic structure?

If a solution of gelatin containing a trace of K_2CrO_4 is poured on a piece of glass and allowed to set and then a drop of silver nitrate placed on the gel, there will result a precipitation of red silver chromate as the silver solution diffuses into the gel. This precipitate contrary to expectation is not continuous but is laid down in rings concentric to the drop of silver nitrate. This ring formation is the periodic structure which Hatschek refers to above and the example given is the classical experiment of Liesegang after whom the phenomenon is sometimes named.



The illustration is a photomicrograph of the formation of the rings of silver chromate in gelatin which I prepared. The large clear zone in the center surrounded by a dark ring about ¼ inch wide is the space occupied by the drop of silver nitrate. The dark rings concentric to this are the bands of red silver chromate.

Liesegang (1) reported his investigations in 1896 and Wilhelm Ostwald (2) having inspected these "astonishing" preparations advanced the first theory or explanation of this phenomenon.

Ostwald's theory is based on supersaturation, reaching a metastable limit, followed by precipitation. Using the example given above, this theory assumes that the entering silver ion forms with the chromate ion present in the gel, a supersaturated solution of silver chromate. The rate of nuclear formation is lowest where the concentration is the lowest. The concentration of the silver chromate varies inversely with the depth of penetration, hence the nuclei are fewer at a greater distance while immediately outside the drop of silver nitrate the concentration is the greatest and the rate of nuclear formation is the greatest and precipitation results here. The silver ions and the chromate ions which were in advance now diffuse backward to this zone of precipitation. The area which these ions have vacated is now free from any silver ions and chromate ions. The silver ions

(1) Liesegang, Photo.Arch.,321 (1896).
(2) W. Ostwald, Z.phyz.Chem., 27, 365, 1897.

having greater osmotic pressure due to concentration behind them, diffuse faster through this barren zone and meeting the chromate ions again form a supersaturated solution and the above process is repeated and there is formed a band. This is repeated many times, the bands or rings being separated by regions of clear gelatin.

Hatschek (1) objects to this theory on the grounds that periodic precipitation takes place in the presence of crystalline nuclei which should prevent supersaturation.

Liesegang (2) in spite of Hatschek's objection to Ostwald's theory believes that supersaturation plays an important part.

Freundlich (3) also accepts this theory with the slight modification that the substance present in supersaturation solution is adsorbed by the colloidal particles which influence the nature and rate of nuclear formation.

This hypothesis has also been supported by Morse and Pierce (4) who attempt a mathematical analysis and by Stansfield (5), Holmes (6), Lord Rayleigh (7) and Notboom(8).

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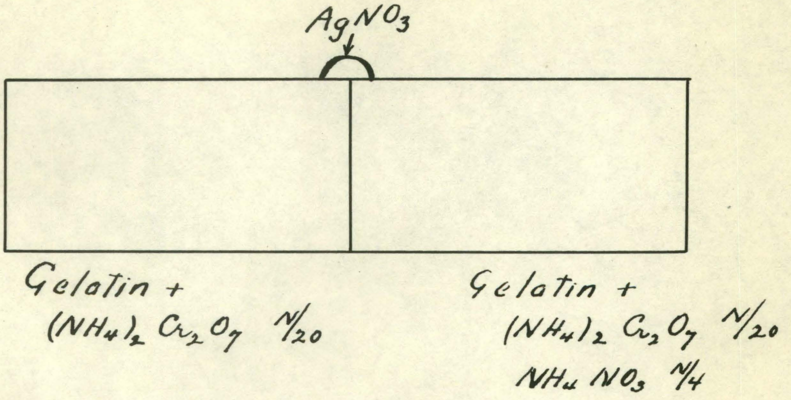
(1) Hatschek, J.Soc.Chem.,Ind. 30.
(2) Liesegang, Colloid Chem., J.Alexander, p.787.
(3) Freundlich, Elements of Colloidal Chemistry, by Freundlich.
(4) Morse and Pierce, Physical Review, 43, 1, 1903.
(5) Stansfield, Amer.J.Sc., 43, 1, 1917.
(6) Holmes, J.Amer.Chem.Soc., 40, 1187, 1918.
(7) Lord Rayleigh, Phil.Mag., 38, 738, 1919.
(8) Notboom, Z.Phys.Chem., 52, 185, 1905.

Bechhold (1) after considerable experimental work put forth in 1905, the theory that the precipitate of the band is slightly soluble in the products of the reaction and hence new bands can form only in regions where the concentration of the reaction by-products is so dilute that it will not interfer with the precipitation.

An example of his work which supports this theory is as follows. One block of 5% gelatin made N/20 with respect to $(NH_4)_2\ Cr_2O_7$ is placed adjacent to another block of 5% gelatin also N/20 with respect to $(NH_4)_2Cr_2O_7$ and also containing $(NH_4)\ NO_3$ to make the solution N/4. A drop of $AgNO_3$ is placed at the junction of the two blocks as shown in the illustration.



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(1) Bechhold, Z.phys.Chem.,52, 185, 1905.

The rings which formed in the block containing the ammonium nitrate were wider and slightly closer together than in the other block. At greater distances the effect is more pronounced.

Bradford (1) using more refined methods was unable to confirm Bechhold's work. A protocol of Bradford's experimental work is:

Series of test tubes #1 containing 20 cc agar gel N/20 Na_2CO_3
" " " " #2 " " " " " " " "
and made N/20 NaCl.

5 cc of dilute $CaCl_2$ was added to each tube. Diffusion proceeded at the same rate in each tube with the formation of bands at distances below the surface of the gel in mm as follows:

Series 1	20.5	22.5	25	28	32.5	35.5
" 2	20.5	22.5	24.5		33.0	35.5

It can readily be seen that the reaction by-product NaCl in series #2 has no effect on the penetration and formation of bands. Bradford decided therefore, that the effect of the by-products is negligible.

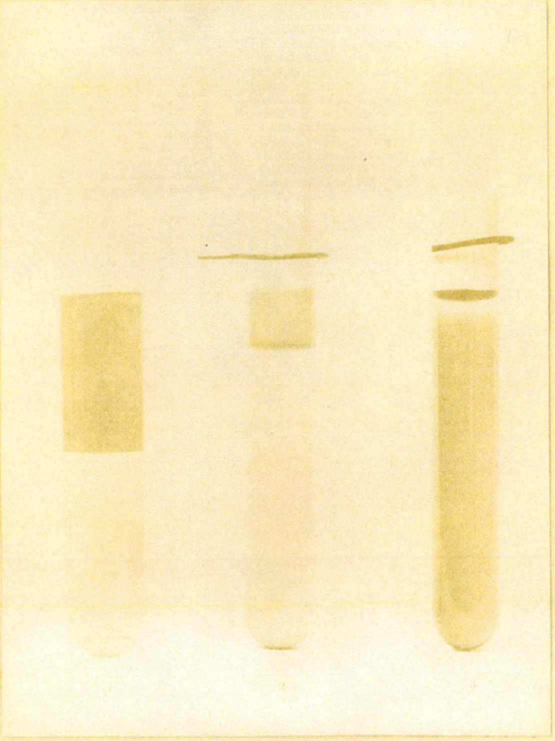
(1) Bradford, Biochem.J., 14, 29, 474, (1920)

Bechhold must later have abandoned his theory for he makes no mention of it in his book (1) several pages of which are devoted to this phenomenon.

Furthermore, he gives some of his experiments in this book which would oppose his earlier theory. He dissolved some globulin in a gelatin solution containing sodium chloride. Water was layered over the gel and the sodium chloride diffused out of the gelatin into the water leaving the globulin precipitated in uniform turbid layers in the gelatin. He concludes that this phenomenon of periodic stratification is not dependent on the interdiffusion of two solutions as was essential in his earlier theory.

(1) Colloids in Biology and Medicine, H.Bechhold, 1919

The following plate is taken from Bradford's article in which he shows that a precipitate or solid matter suspended



Proof of Adsorption.

#1 Lead chromate in Agar gel.
N/20 potassium chromate upper solution.

#2 Fuller's earth in 1% Agar over the same gel with a colored dyestuff after a few days.

#3 Catalpo No. I and Magento in 1% Agar after 17 days.

over a gelatin gel containing a colored salt acts to remove the colored salt from the gel by a process of adsorption on the surfaces of the solid particles.

Microscopic examination of the formation of the precipitate in a gel revealed to Bradford that the particles were spherites, globular crystals, with radiating needles indicating that these particles have enormous specific surface.

Consideration of these facts (a) that the hypotonic reagent is removed from the spaces below the precipitate and (b) that only precipitates which are either colloidal or extremely finely divided separate into strate, led Bradford to the conclusion that it is the adsorbing action of the large surface of the precipitate which removes the solute from the layer of gel in its vicinity so that the hypertonic reagent, diffusing through has to traverse an exhausted zone of gel before meeting with sufficient solute to produce a new band.

The adsorption theory met with the objection that it took no account of the influence of the gel. Silver chromate which formed in stratifications in gelatin gels did not do so in agar or in silica gels. Bradford explains the different effects obtained with the same insoluble substance in different gels as due to the influence of the gel or reaction medium on the factors K and P in von Weimarn's formula which determine the specific surface of the precipitate and therefore, its adsorbing power. By application of von Weimarn's theory it is possible to obtain silver chromate or dichromate strata in agar.

Bradford's modification of von Weimarn's formula is as follows:

$$N = V\frac{P}{L}$$

where N is the number of crystallization centers.

V is a function yet undetermined of the viscosity of the medium and of the size and structure of the particles in solution whether due to physical aggregation or chemical complexity. It is an incomplete expression of the truth but it is useful as a working rule.

Band precipitation occurs only when the specific surface of the precipitate is great enough to cause exhaustion of the solute. Decrease in N leads to diminution in absorbing surface until stratification ceases. Increase in N may be accompanied by an increase in density of the precipitate or slower diffusion so that the precipitant cannot permeate the precipitate fast enough to traverse the exhausted region before diffusion has occurred from below filling the exhausted zone with the solute.

Since the solute in the gel must be sufficiently dilute to allow of its exhaustion from the neighborhood of the precipitate with corresponding limitation of the value of P it is clear that stratification can take place only when L is very small or when K*is large enough to compensate for a somewhat larger value of L.

*K is replaced by V in the modified formula.

Reaction medium by its viscosity and protective action profoundly modifies the value of K and P and thereby the specific surface.

Bradford's theory was probably the best which had been offered. It has met with the objection that where silver nitrate and potassium chromate meet each other and react, there is no solid silver chromate present at the beginning but only dissolved silver chromate in the state of supersaturation. Therefore, it is hard to believe that an adsorption of potassium chromate on the silver chromate takes place in this region.

Dhar and Chatterji (1) also find it difficult to accept Bradford's account since they find that freshly precipitated silver chromate hardly adsorbs any potassium chromate even from a dilute solution.

Also in my own work I have frequently observed that mere diffusion of the colored salt out of the gel would present a picture similar to that on page

(1) Dhar and Chatterji, J.Phys.Chem.,28, 41, 1924.

In chronological order the next explanation of periodic precipitation is that of Williams and MacKensie (1). They have determined very carefully the solubility of silver chromate in gelatin and found it to be soluble to the extent of 0.069 parts per 100 cc of gelatin which is about 25 times more soluble than it is in water at 20°C. "From the assumption that the protected silver chromate might be functioning as a colloid, it seemed reasonable to expect general precipitation to follow diffusion of either silver or chromate into a gel containing the maximum amount of silver chromate. Consequently, a series of tubes were half filled with 3% gelatin made N/240 with respect to silver chromate. The gel was allowed to set and then solutions of silver nitrate and potassium chromate were poured above the gel.

N/250	Silver Nitrate	-reddish haze 2-3 cm.below gel surface.
N/200	" "	-intense reddish haze 1.5-2.5 cm "
N/100	" "	-traces of bands at 0.5,1.0,1.5 cm.
N/50	" "	-bands at 0.2,0.5,0.9,1.4,2.1,3.2,haze at 4.
N/10	" "	-fine bands from 0-5 cm.
N/2	" "	-thicker, closer bands from 0-4.5 cm.

N/250	Pot.Dichromate	-haze from 0.5-2.5
N/200	" "	-haze from 0.5-3.0
N/100	" "	-thick bands from 0.5,trace at 0.9.
N/50	" "	-thick bands above 0.2,0.5,0.8,1.5
N/10	" "	-thick bands 0.3,0.6,0.9,1.5,2.3
N/2	" "	-general precipitation with banded structure visible 0-4.9

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(1) Williams and MacKensie, J. Chem.Soc.,117, p.848,1920.

In all cases, there was a precipitate at the actual interface. It will be seen that even under the conditions given, stratification took place. This means that in some manner or other, the gel beneath a stratum of precipitate was cleared of the silver chromate, whatever the cause, so to clear a preceptible space, diffusion must have occurred. In other words, the silver chromate did not function as a colloid with a slow rate of diffusion."

As a result of the work they have reached the following conclusions. The precipitation of silver chromate occurs according to the usual rules of the solubility product. By diffusion towards the precipitate the next portion of the gel is cleared of the second ion more quickly initially than the precipitating ions can reach it in quantity, thereby setting up a concentration gradient so that these ions must travel some distance before further precipitation takes place. It is obvious that the gel incidently provides mechanical support for the precipitates.

"The distance at which a second stratification appears is evidently dependent on the relative rates of diffusion of the two ions. The diffusion of the first ion may be hindered by the precipitate or by precipitating at the precipitate. The faster the second ion diffuses to the precipitate the further from the precipitate will be the mean value of the concentration in the region of diffusion and hence under suitable conditions (that is, not too high concentration of one reactant, for example, potassium chromate behind the precipitate, the further from the precipitate will be the place at which the new precipitate forms."

"Diffusion is a slow process and in the case considered can only be accelerated by reducing the concentration to which the diffusion proceeds, that is, the concentration at the precipitate. Bradford assumes this reduction is accompanied by adsorption (see under Bradford) but although adsorption at the surface of the adsorbent is faster than diffusion, it cannot accelerate diffusion otherwise than is here indicated, and since simple continuous precipitation at the stratum would have the same effect it is unnecessary to assume adsorption."

Dhar and Chatterji (1) in 1924, after critically reviewing the existing theories accounting for the Liesegang phenomenon and as a result of their own experimental work have developed the following explanation of this occurrence based on peptization followed by coagulation. If lead nitrate is permitted to diffuse into a gel containing potassium chromate the lead nitrate which is formed is peptized (and is therefore colloidal) and protected by the gel. The concentration of lead chromate gets beyond the protective power of the gel and there results a coagulation of lead chromate; this process of coagulation is assisted by the presence of

(1) Dhar and Chatterji, J.Phys.Chem.,28-47, 1924.

the reaction by-product, potassium nitrate. The lead nitrate penetrates this coagulum and meets the potassium chromate but only at first such quantities of lead chromate are formed as can be protected by the gel. Due to the influence of the solid lead chromate above, these ions migrate to it leaving a clear zone thru which the lead nitrate must penetrate in order to reach the potassium chromate and form another coagulum.

The previous work of Williams and MacKensie must necessarily oppose this explanation since among other things, they conclude that in their work the silver chromate did not function as a colloid, as Dhar and Chatterji claim, with a very slow rate of diffusion but as a crystalloid. Dhar and Chatterji, however, feel that the work of Williams and MacKensie is open to some criticism and objection and therefore will not accept it.

J. Alexander criticizes on this work by saying that an ultra fine precipitate which, though colloidal, should possess considerable diffusibility. Very recently Chatterji and Dhar (1) have measured the conductivity of silver chromate in gelatin and find that it is considerably less than if the silver chromate were in true solution. Hence they say it is colloidal.

(1) Chatterji and Dhar, Trans.Farad.Soc.,p.23, Jan.,1927.

Holmes, who has investigated periodic precipitation and crystallization in silicic acid gels to a very large extent put forth the following explanation in 1918 (1) which is based on ionic activities.

A slightly basic silicic acid gel containing a small amount of a chromate was poured into a test tube. Over it was layered a solution of a copper salt. The /Cu ions diffuse down into the gel, meet the chromate ions and a layer of copper chromate precipitates out. The chromate ions below this layer diffuse into this region now exhausted of chromate ions and meet the incoming Cu ions thus thickening the layer of precipitate. According to Fick's law of diffusion, the rate of diffusion is greatest where the difference in concentration of the chromate ions in two contiguous layers is the greatest, that is, just below the front of this thickening band of copper chromate. As a result, the region just below the band decreases in concentration of the chromate ions faster than does the space below. Finally the copper ions have to advance some distance beyond the band to find such a concentration of chromate ions that the solubility product may be exceeded and a new band formed. This repeats again and again.

As one instance in favor of this theory Holmes cites Liesegang's (Z.angew.Chem.,23, 2124, 1910) "dead space"

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(1) Holmes, J. Am.Chem.Soc.,40, 1187, 1918.

experiment. More will be said of this later in the experimental work wherein results different from Liesegang's were obtained.

The same objection which Hatschek makes to the explanations of Bradford and Ostwald that these theories leave the specific effect of the gel entirely out of account, may also be applied to this theory of Holmes.

Bancroft (1) says of this theory that it "unfortunately does not account for the fact recorded in the same paper, that colloidal gold gives three colored bands, red, purple and blue, before repeating."

(1) Bancroft, Applied Colloid Chemistry, p.259,by W.D.Bancroft,1921

McGuigan and Brough (1) after reviewing the various manifestations of periodic structure have arrived at the conclusion that all chemical action may be periodic; e.g., Ostwald (2) found that if the rate of evolution of H gas, when HCl acts on one of the allotropic forms of chromium, during the action, be plotted as ordinate against time as abscessa, the curve will show alternating periods of rapid and slow evolution. The curve in a measure corresponds to ring precipitation.

They summarize their conclusions as follows. "A theory of rhythmic precipitation is advanced, which accounts for the action of the gel. This theory accepts the descriptions of the process given by Bradford and by Holmes, which are essentially alike. Precipitation in gels does not differ from precipitation in water; therefore, adsorption is no more important in gels where precipitation is banded, than in water where it is continuous. The function of the gel is to fix the precipitate where it is formed, and to restrict the zone of the influence of the ions, by reducing to a minimum the factors which make for turbulence or diffusion. It is pointed out that any influence that lessens the turbulence of the reaction, makes for rhythmic precipitation."

(1) McGuigan dnd Brough, J.Biol.Chem.,58, 423, 1924.
(2) Ostwald, W., Z.physik.Chem.,35, 33, 204, 1900.

They object to Bradford's theory on the grounds that this work could equally well be used to support any of the other theories.

Adsorption cannot be a prime factor in initiating or controlling the reaction since surface energy always exists sometimes negative and sometimes positive and banding may occur in both conditions.

The influence of adsorption would also seem much less than that of the ion, since adsorption varies inversely as the fourth power of the distance, while chemical attraction varies inversely as the square of the distance.

The theory of Dhar and Chatterji meets with very strenuous objection by Wo.Ostwald (1). Wo.Ostwald has been able to form banded precipitates in water and therefore, concludes that the gel and the formation of colloid particles is not essential for the formation of rings but is merely an accessory factor.

In turn Wo.Ostwald (2) presents his "Diffusion Wave Theory" of Liesegang rings. This proposed theory refers only to rhythmic precipitation resulting from chemical reaction in which the components diffuse toward one another. It is based on the following ideas: (a) in all reaction systems which produce typical rhythmic precipitations at least three principal diffusion waves are formed and interfere: (b) many, perhaps all, typically rhythmic precipitations belong in the sense of the mass action law, to the so-called "limited reactions", e.g., in contrast with such reactions as the precipitation of barium sulphate, they are incomplete. The diffusion of ammonium hydroxide into gel containing magnesium chloride is considered typical of such reactions as silver nitrate and potassium dichromate. At first the diffusion wave of ammonium hydroxide into the gel is retarded by the formation of magnesium hydroxide which keeps the concentration and the diffusion gradient of the ammonium hydroxide in the gel small. The magnesium chloride sets a diffusion wave towards the ammonium hydroxide,

(1) Wo.Ostwald, Kolloid Zeit., Oct.,1924, p.144.
(2) Wo.Ostwald, Kolloid Zeit., Special Number,April 1,1925,p.380.

i.e., the region of lowest concentration. At the same time, the diffusion gradient of the reaction electrolyte increases rapidly (2 NH_4Cl are formed per $MgCl_2$). The ammonium chloride diffuses in opposite directions from a maximum concentration at the zone of reaction where the concentration of ammonium hydroxide and the magnesium chloride is kept so small that the precipitation of magnesium hydroxide is prevented in accordance with the mass action law. The following experimental observations are given in support of this theory.

(1) All rhythmic precipitations dissolve in an excess of the reaction electrolyte, i.e., rings of magnesium hydroxide are dissolved if an excess of ammonium chloride diffuses into the gel.

(2) The width and distance of the bands are changed by previous addition of the reaction electrolyte. Continuous precipitates were decomposed into rings and inversely rings disappeared by the extension of the initial precipitate, e.g., adding ammonium chloride to a gel containing magnesium chloride before the ammonium hydroxide enters by diffusion.

(3) If one component is varied in the sense of the mass law, the precipitation becomes continuous.

(4) Metastable limits, peptization and coagulation, adsorption on the precipitates, etc., are not considered fundamental conditions for rhythmic precipitation. These are secondary factors insofar as they influence the form, position and velocity of diffusion waves.

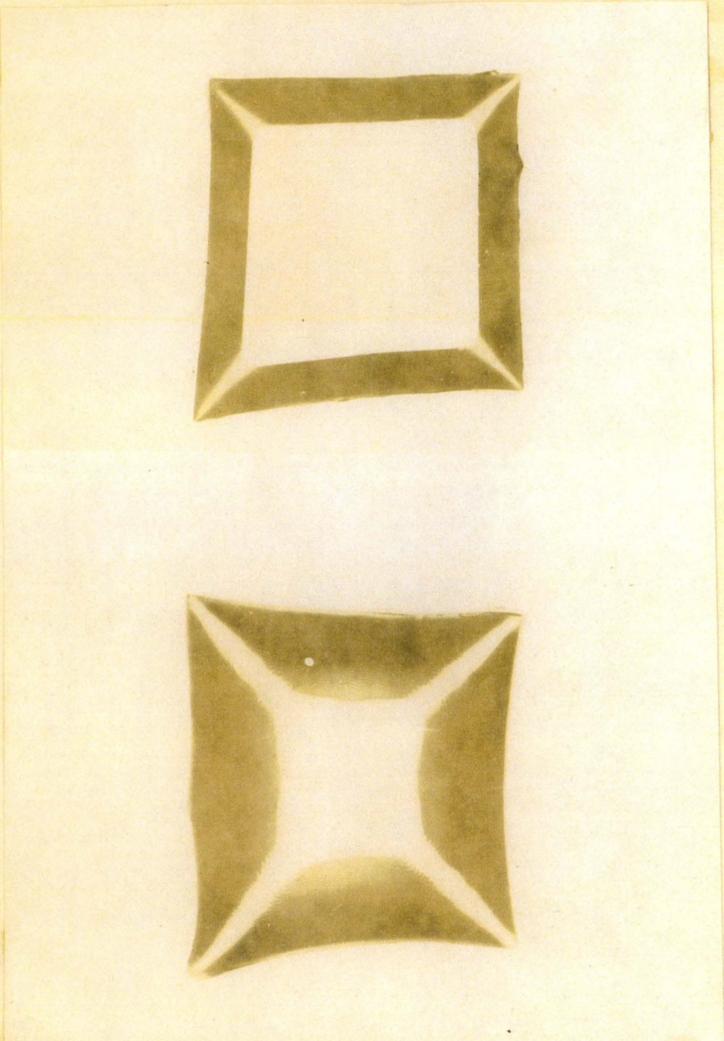
It is interesting to note that Wo. Ostwald is the first to differentiate the various types of periodic precipitation and that his theory deals only with such reactions in which the ions diffuse toward one another.

An analytical study of his theory left me in doubt as to the real reason for periodicity. He does, however, account for the action of the gel, etc., in a very satisfactory manner. I have been unable to confirm his work on the influence of the reaction electrolyte on the formation of rings.

Section II

I. Microscopic examination of the formation of the pattern and bands of lead chromate in a block of gelatin.

Riegel and Widgoff (1) report the formation of a pattern containing fine bands of lead chromate in a gelatin cake. A section of such a cake which I prepared is shown in the illustration.



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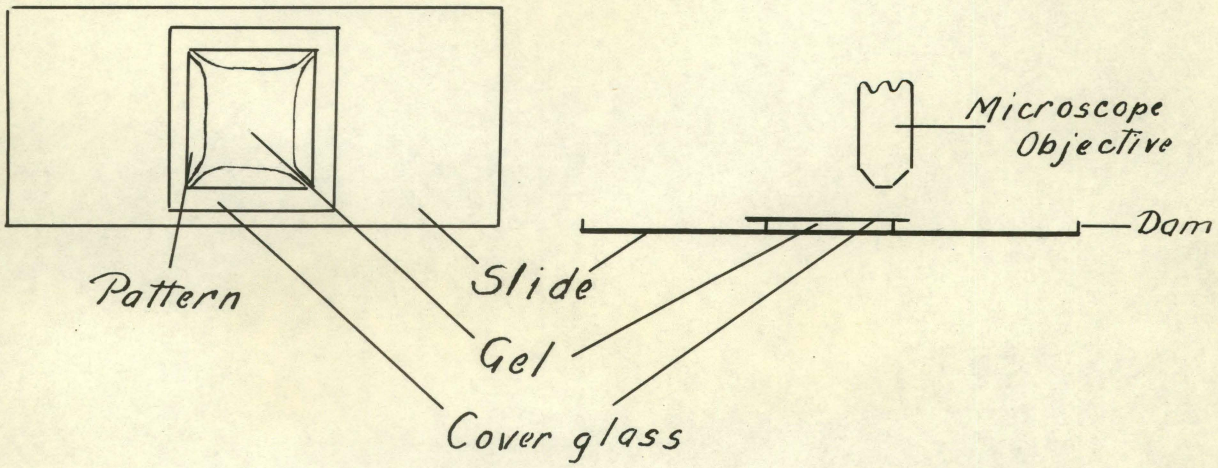
(1) Riegel and Widgoff, J. Phys.Chem.,29, 872, 1925.

It can readily be seen from this picture that the corners of the cake are free from any precipitate and that the bands start abruptly at the edge of this clear zone.

It would be of great interest to watch the actual formation under considerable magnification of this structure.

A cake of 4% gelatin containing potassium chromate (0.1 g in 100 cc of gelatin) was made up and sectioned into slices 1 mm. thick and 15 mm. on each side. A slice was mounted on a glass slide and covered with a cover slip, care being taken that the gelatin made intimate contact on its upper and lower surface with the glass. Lead nitrate (1 gram in 100 cc of water) was flowed around this section of gelatin. By this arrangement the lead solution penetrated the gelatin under the same conditions approximately as it did into a cake immersed in the solution.

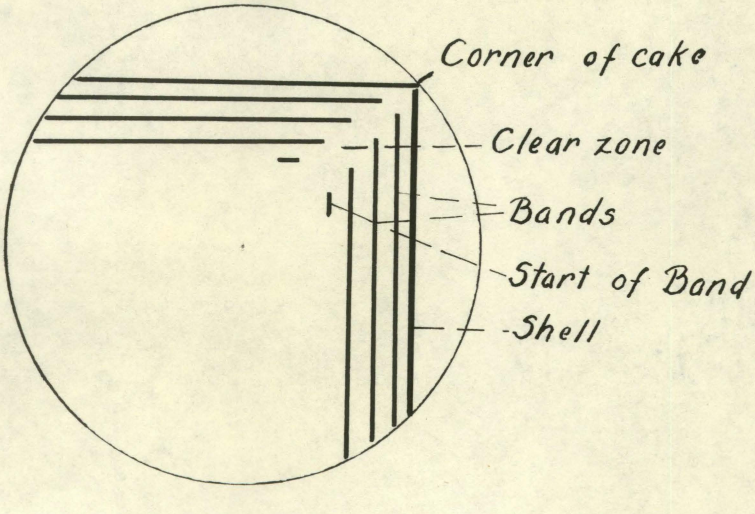


The glass slide was then placed on the stage of the microscope and so adjusted that the low power objective was focused on the side or edge of the cake.

Observation through the microscope revealed the following occurrences. At the surface or edge of the gel there appear a few small yellow dots which gradually increased in number forming a dense precipitate. For a short period of time there seemed to be no further action when in at a short distance from the precipitate toward the center of the cake there again appeared a few dots which increased in number forming a dense structure within narrow limits. The gel between the outside precipitate and this narrow structure contained but a few particles. This narrow structure which macroscopic examination showed to be a band, was densest at the center and thinner on either side eventually fading away to clear gelatin. Again for a short period of time nothing happened when at a slight distance in from this band there again appeared a few particles which eventually formed a second band.

During the first minute of reaction three such rings formed, three more during the next minute. The rings which had formed first seemed to have merged into a solid outside structure or shell. As the rings progress towards the center of the cake the distance between each ring becomes greater and the formation slower than at the periphery.

The slide was moved so that the corner of the cake came under the objective. The field was sufficiently large to take in the clear zone which is evident in the illustration as well as the precipitate on both sides of the clear area. At the time of observation several bands were present. Continuous observation revealed that after one band is complete, the next band which is laid down, as previously described as a few particles which increase in number, started to form at the edge of the clear area and grew parallel to the side of the cake. This occurrence repeated itself for every band on both sides of the clear zone, the band starts at the clear zone. The illustration pictures this.



This phenomenon suggests that the entering ions penetrating at right angles to the four surfaces exposed form with the ions in the gel, a precipitate at the outer edge of the cake which impedes or slows down the entering ions sufficiently that the ions coming in at the corners move a greater distance and so reach the zone of reaction just slightly before the ions entering from the side. Hence the first band or ring starts at this corner or clear zone. Subsequent rings are formed in the same manner, all of which impede the progress of the ions entering at right angles to the surface of the gel.

Miss Foster (1) reports an occurrence similar to the above in some respects. She followed the formation of the bands of silver chromate in gelatin and found that the growth of the bands was lateral, she however, made no observations of the point of origin of the bands.

(1) Foster, J.Phys.Chem., 23, 645, 1919.

II. A microscopic study of the formation of the bands and pattern of silver chromate in a gelatin cake, with some consideration of the time factor.

Riegel and Widgoff (1) also report the formation in gelatin of a zone pattern with bands of silver chromate. The progress in the formation of this salt was studied in the present work as follows.

A cake of 5% gelatin containing 0.1 g. of potassium chromate per 100 cc was sectioned and mounted as described in I. One % silver nitrate was flowed around the gelatin. Intermittent examination by means of the microscope, of the precipitate while forming revealed the following essentials.

After two minutes of reaction there had formed at the edge of the gelatin cake fine white hair-like lines. After 15 minutes the hair lines had advanced toward the center of the cake and there were also four dense red bands superimposed on the fine lines in addition to the dense outer red "shell". About 20 hair lines preceded the last red band. After another 14 minutes three more red bands had appeared, the white hair lines still in advance towards the center of the cake. The next two bands which were the last to form took about ten minutes a piece to form. The hair lines nearer the center of the cake were wider and farther apart and the particles were larger than at the periphery of the cake.

(1) Riegel and Widgoff, J.Phys.Chem.,29, 872, 1925.

Finally the hair lines which were subsequently laid down were not distinct, giving way to a cloud of particles without structure or design.

The observation of the actual laying down of a red band is: After one red band is complete there appears after a short interval a gradual darkening with red of the fine white hair lines about 6 or 7 lines ahead of the completed red band. This darkening continues until it is difficult to distinguish the individual hair lines. Those hair lines at the center of the red bands are the densest.

The illustrations on page 36 are a series of photomicrographs of the cake showing the zone pattern and red and white structures.

#1 is a picture of a section of the whole cake showing the zone pattern.

#2 is a photomicrograph of the lower left corner of #1.

#3 is a series of individual photomicrographs of a section of a similar cube starting at the exterior of the cake and following through to the center. It was taken through the region as indicated by the arrow in #2.

#4. Two photomicrographs of the corner of the section showing that the bands and hair lines are continuous at the corners.

Since as is shown that the bands are continuous at the corners although not as dense, no observations as to the point of origin of the bands could be made as in #1.

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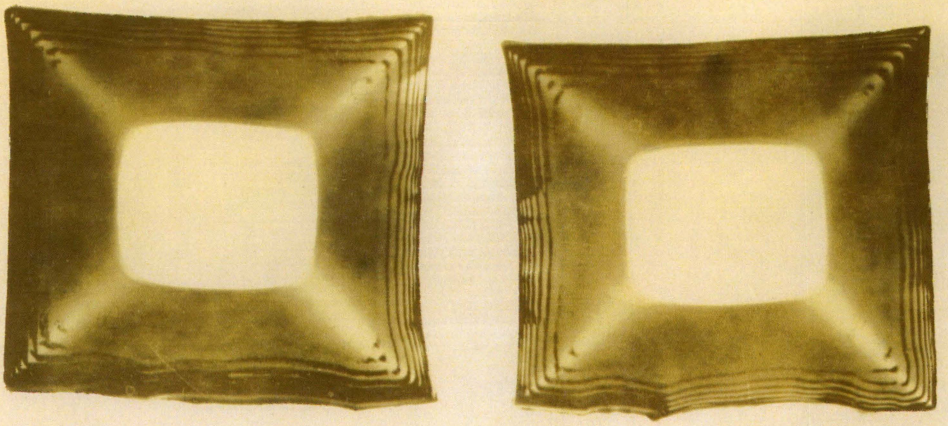

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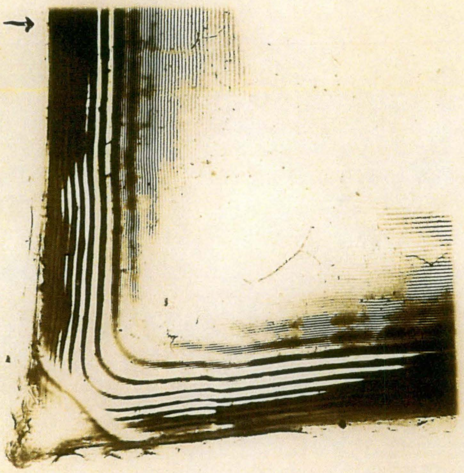

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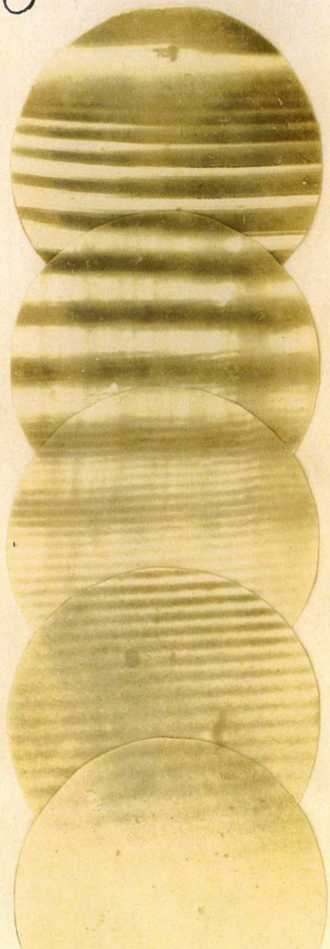

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III Microscopic study of the silver chromate formation in a test tube.

In as much as in the two previous investigations the difficulties in obtaining sections of uniform thickness were so great, frequently only one section out of ten could be used, that it was decided upon to extend the microscopic examination by a different method.

For this purpose special test tubes were constructed which would lend themselves to ease of handling and examination both macroscopically and microscopically. Flat glass tubing 18 mm wide and 7 mm thick was obtained in sections 12 cm long. These were sealed closed at one end. To the other end was attached ordinary round tubing. The flat part was 3/4 filled with the gelatin solution. The reaction liquid poured on top and a stopper inserted into the round part so as to exclude any air bubbles. Thus the tube could be laid horizontally or inverted without separating the reaction liquid from the surface of the gel.

As a preliminary study with this method a five per cent gelatin containing 0.1 g potassium chromate to 100 cc of gelatin was used, with twenty per cent silver nitrate poured on top. The tube was placed horizontally on the stage of the microscope and the reaction observed as follows: A dense red precipitate formed immediately at the interface, subsequently I will term this first dense precipitate as the shell. In four minutes the fine yellow-white hair lines were visible ahead of the shell. After nine minutes there appeared to be a red band forming in advance of the shell. This band made its first appearance merely as a darkening with red of the hair lines in advance of the shell at one side of the field under observation. This darkening gradually grew across the field and formed a definite red band. Following the completion of this band the intervening space between it and the shell was filled in with red forming a solid red, bandless mass continuous with the shell. Evidently the shell forms as a band laid down rapidly and subsequently merged. During the next two minutes similar additions to the shell occurred but not as rapidly or as pronounced. About 20 hair lines always preceded the shell.

Twenty minutes after the start of the experiment it took seven minutes for one hair line to cross the field, a distance of 1.2 mm.

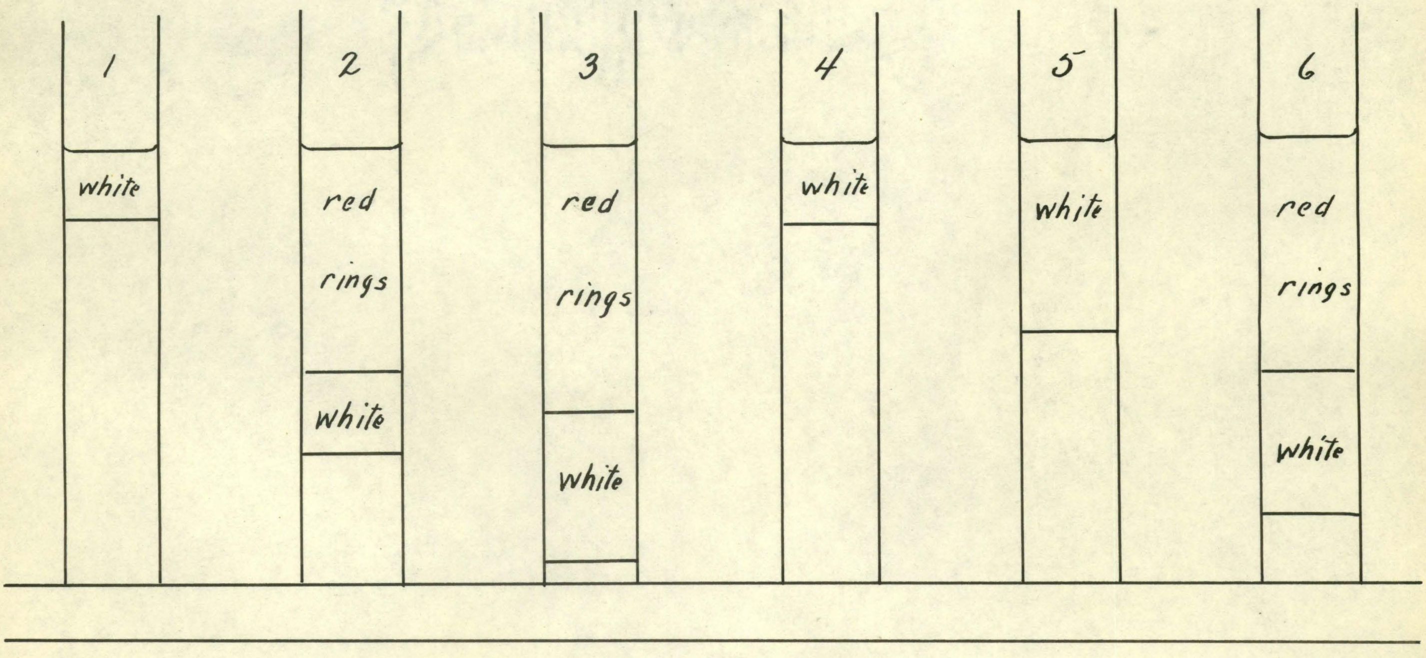
No further observations were made on this tube. This preliminary trial showed that this method is superior to the former and furthermore, admits of subsequent permanent recording as a photograph or drawing which the other did not.

An interesting observation was made during this study which is worth noting. A red precipitate is not laid down in definite particles but apparently is merely a darkening of the existing white hair lines which are composed of small particles or of the gelatin in that region.

IV. Further microscopic study of the reaction in a test tube, also a study of the effect of varying the concentration of the three components of the system, namely the gelatin; the inside solute, potassium chromate; and the outside solute, silver nitrate.

The flat tubes were handled as described in the previous work. The following drawing shows the concentrations used and the resulting penetrations and formations.

	1	2	3	4	5	6
Gelatin	10%	10%	5%	5%	5%	5%
$AgNO_3$	0.1%	1%	1.0%	.05%	.01%	1%
$K_2Cr_2O_7$	0.1%	0.1%	.05%	.05%	.05%	0.1%



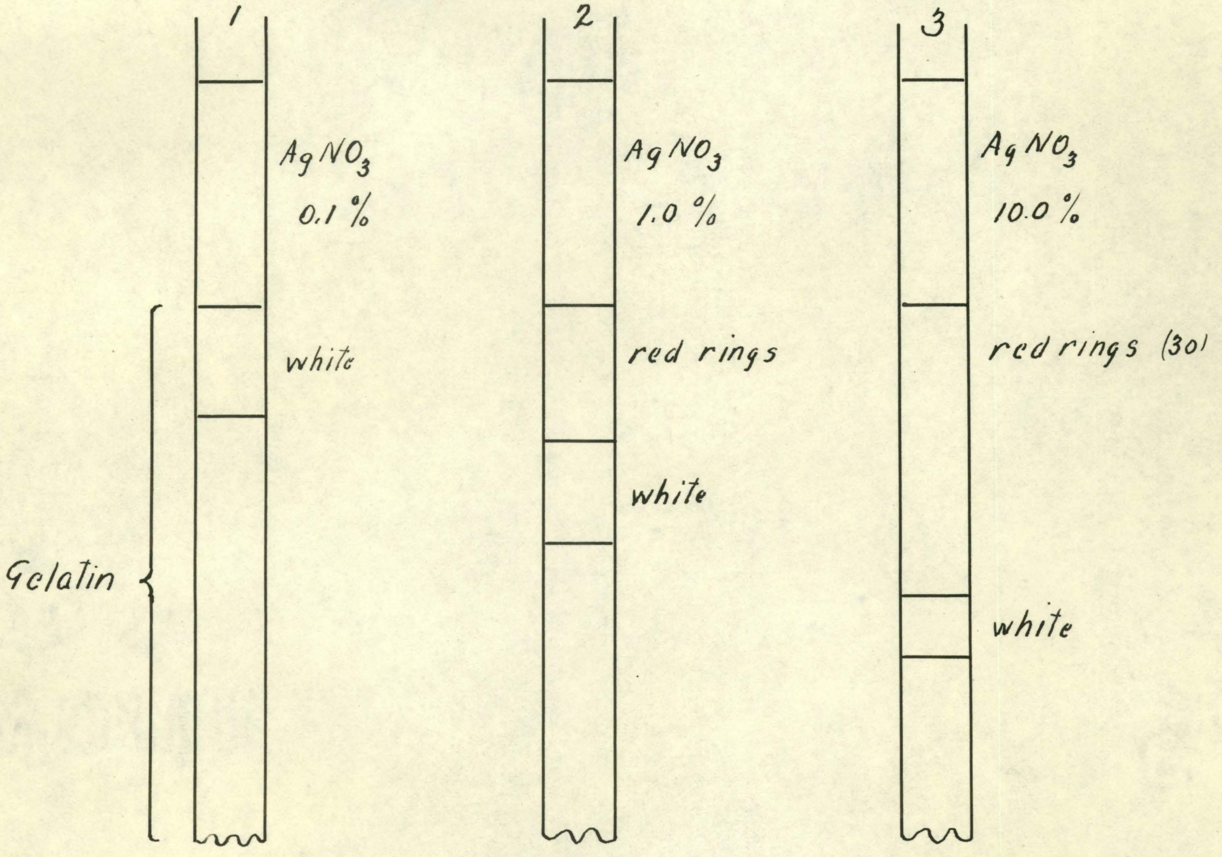
From this chart it can readily be seen that a comparison of #2 and #6 in which the gelatin is the only variable, the effect of increased concentration of gelatin decreases the total penetration as evidenced by the precipitation. In numbers 1, 4 and 5 in which the silver solution is dilute there is no red precipitate, an explanation of this will be given later. With smaller amounts of dichromate the total penetration was greater for both red and white precipitate.

Microscopic examination of these tubes while the reaction was going on showed that in those tubes (2, 3 and 6) which had a red precipitate the formation of the shell was the same as in the preceding experiment. A red band would become superimposed on the white hair lines in advance of the shell. The intervening space would be filled in with red precipitate forming a continuous structure.

In all of the tubes the white portion showed hair lines. In numbers 1, 3, 4 and 5 there were denser zones in the white portion.

In the previous work the inside measurements of the tubes varied considerably so that accurate observations on the penetration could not be made. The following experiment was therefore, carried out with round glass tubing of uniform inside diameter (9 mm). Three tubes were filled with equal quantities of 5% gelatin containing 0.1% potassium dichromate. In these tubes 1, 2 and 3 were placed equal amounts of 0.1% , 1.0% and 10% silver nitrate solutions respectively.

The following chart drawn very carefully to scale shows the results after 42 hours of reaction.



Scale X1

The following chart shows the progress of the penetration and the resulting formations.

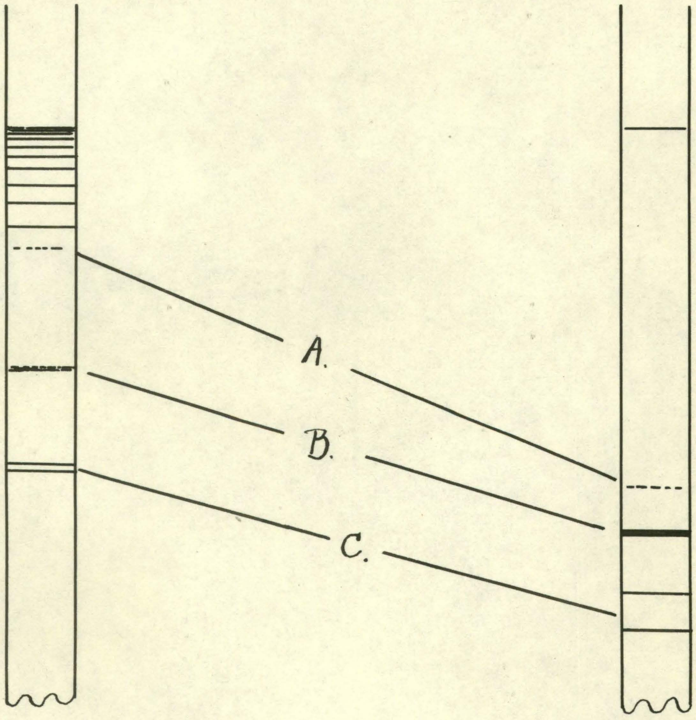
Duration of Reaction.	#1 - 0.1% $AgNO_3$	#2 - 1.0% $AgNO_3$	#3 - 10% $AgNO_3$
After			
1 min.	white film	red film	dense red film
4 min.	" "	red and white film	red film only
9 min.	" "	" " "	red film and slight white under red
50 min.	2½ mm. all white	4-5 red rings in first 3 mm. 2 mm.of white below this	very fine red rings at top. 5½ mm.of red 1½ mm.of white
1½ hrs.	3 mm.white	4½ mm.red 2½ mm.white	8½ mm.red 1½ mm.white
42 hrs.	14 mm.all white with small red spots at interface between liquid and gelatin.	11 red rings occupying 13 mm. 18 mm.of white haze	30 red rings in first 35 mm. 11 mm.of white.
50 hrs.	15 mm.all white except as noted above	13 mm.red 18 mm.white	51 mm.total penetration. 8 mm.of which are white.

Evidently reaction is approaching an end.

We can conclude from these results that the effect of increased concentration of the silver nitrate is to increase the number of red rings, the total penetration and to decrease the white precipitate.

Since in these tubes the reactions apparently were approaching an end they were placed in the refrigerator in order to preserve them. After two days they were again examined and very surprisingly, new structures had formed which were very different from the existing formation.

In tubes 2 and 3 considerably beyond the last red precipitate or beyond the region where another red ring might be expected to form there was a thick red zone much denser and more compact than the last ring which had formed at room temperature. A white haze formed a definite zone beyond this ring which also was much denser than in the haze formed at room temperature.



A. Last ring formed before putting in refrigerator.
B. Rings formed in regrigerator which are denser than A.
C. Dense white precipitate formed in refrigerator.

The subsequent unexpected ring formation can be explained on the assumption that there existed in the gelatin, silver chromate in true solution beyond the last red ring. (Silver chromate is soluble to the extent of .0028 parts in 100 cc of water at 18°C). Lowering the temperature of the solution merely decreased the solubility of the silver chromate and as a result it precipitated out and formed a ring.

If this salt had existed in a supersaturated solution as Ostwald claims, it seems reasonable that in the long period before the tube was placed in the refrigerator it would have migrated back to the last ring and precipitated there.

Dhar and Chatterji assume that the particles of silver chromate exist in a colloidal form before precipitating. This does not seem satisfactory in view of the above result since it seems to me to be more reasonable to conclude that lowering the temperature would increase the stability of a colloidal solution and so prevent precipitation which is contrary to the above.

V. A study of the influence of time and temperature on the formation of silver chromate in a gelatin cake.

Cubes of 5% gelatin containing 0.1% potassium dichromate were made up to be fairly uniform in size, namely 2 cm. on an edge. These were immersed in equal portions of 1% silver nitrate solution. One half of these immersed cubes were placed in a refrigerator at a temperature of 3°C, the remaining cubes were left in the room at a temperature of 20°C. At the end of certain intervals of time as recorded below, the cubes were removed from the solution, a thin section was taken horizontally through the center and mounted on a glass plate and examined under the microscope in the following manner.

The microscope was equipped with a movable stage. The movement was controlled by very fine screws so that very small movements could be made. The amount of movement could be read on a millemeter scale with a vernier attachment which read in tenths of a millemeter. A hair was mounted in the eye piece of the microscope which served as a pointer or indicator.

The section to be examined was clamped on this stage and the glass slide moved so that the indicator when the section was in focus was on the outside shell of the cake. A reading was made on the scale. The stage was then moved until the indicator was on the first band or ring when another reading on the scale was made. The difference between the two readings gave the distance between the shell and the band. Similar readings were taken for all of the bands as well as for the white hair lines and haze near the center of the cake. In this way it was possible to obtain not only the total penetration of the red and of the white precipitate but also to count very accurately the number of rings. It was hoped to be able to measure the distance between the bands and also the thickness of the bands. I was unable to do this, however, because the slice of gelatin was so thick (approximately 1 mm.) that it was impossible to focus sharply on any one part. Also, the bands were not always perpendicular to the axis of the microscope so that the true distance between two bands could not be accurately measured.

The measurements are given below.

Temperature $20^{\circ}C$		$35^{\circ}C$	
Time of immersion 15 min.		15 min.	
Readings		Readings	
20.9 - 20.5	Shell .4 mm thick	23.7 - 23.0	Shell
20.5 - 20.4	Interval.1 mm "		
20.35- 20.3	Band #1 .05 " "	22.95- 22.9	Band #1
20.2 - 20.2	" #2	22.8 - 22.75	" #2
20.1 - 20.0	" #3	22.65- 22.6	" #3
20.0 - 19.9	" #4	22.5 - 22.4	" #4
19.8 - 19.7	" #5	22.4 - 22.0	Hair lines
19.65- 19.5	" #6	22.0 - 21.6	Cloud of dots
19.5 - 18.8	Hair lines, no rings		
18.8 - 18.35	Cloud or haze of dots, no hair lines.		

Penetration.		Penetration.	
Bands 6	1.4 mm.	Bands 4	1.3 mm.
Hair lines	.7 "	Hair lines	.4 "
Cloud	.45 "	Cloud	.4 "
Total	2.55 "	Total	2.1 "

Temperature 20°C	35°C
Time of immersion 30 min.	30 min.
Readings	Readings
21.0 - 20.5 Shell	20.3 - 19.65 Shell
20.5 - 20.45 Band #1	19.6 - 19.55 Band #1
20.4 - 20.35 " #2	19.35- 19.4 " #2
20.3 - 20.25 " #3	19.35- 19.3 " #3
20.2 - 20.1 " #4	19.15- 19.1 " #4
20.0 - 19.95 " #5	19.0 - 18.9 " #5
19.9 - 19.8 " #6	18.75- 18.7 " #6
19.7 - 19.6 " #7 not sharp	18.5 - 18.45 " #7
19.5 - 19.4 " #8 " "	18.45- 17.85 Hair lines
19.5 - 18.0 Hair lines	17.85- 17.3 Cloud
18.0 - 17.55 Cloud	

Penetration		Penetration	
Bands 8	1.6 mm.	Bands 7	1.85 mm.
Hair lines	1.4 "	Ripples	.6 "
Cloud	.45 "	Cloud	.55 "
Total	3.45 "	Total	3.00 "

Temperature 20°C

Time of immersion 60 min.

Readings

22.8 - 22.3	Shell
22.25- 22.2	Band #1
22.1 - 22.05	" #2
22 - 21.9	" #3
21.8 - 21.74	" #4
21.7 - 21.6	" #5
21.45- 21.4	" #6
21.3 - 21.2	" #7
21.05- 18.6	Hair lines

Clouds of dots not pronounced. Cannot distinguish.

Penetration

Bands 7	1.6 mm.
Hair lines	2.6 "
Cloud	?
Total	4.2 "

35°C

60 min.

Readings

22.8 - ?	Shell not sharp
22.2 - 22.1	Band #1
22.0 - 22.0	" #2
21.9 - 21.85	" #3
21.75- 21.65	" #4
21.55- 21.45	" #5
21.3 - 21.24	" #6
21.1 - 21.0	" #7
20.9 - 20.8	" #8
20.6 - 20.5	" #9
20.25- 20.15	" #10
21.15- 19.1	Hair lines. No dots visible.

Penetration

Bands 10	2.65 mm.
Hair lines	1.05 "
Total	3.70 "

Tabulation of Results.

	20°C		35°C	
15 Min.	6 bands	1.4 mm.	4 bands	1.3 mm.
	Hair lines	.7 "	Hair lines	.4 "
	Cloud	.45 "	Cloud	.4 "
		2.55 "		2.1 "
30 Min.	8 bands	1.6 mm.	7 bands	1.85 mm.
	Hair lines	1.4 "	Hair lines	.6 "
	Cloud	.45 "	Cloud	.55 "
		3.45 "		3.00 "
60 Min.	7 bands	1.6 mm.	10 bands	2.65 mm.
	Hair lines	2.6 "	Hair lines	1.05 "
	Cloud	?		
		4.2 "		3.07 "

Remarks:

Examination of this table reveals some interesting points. At low temperature the total penetration is less in every case, this result was of course anticipated. The total penetration as judged by the last red band is greater as well as the number of red rings at lower temperature. This can be explained as the result of the lesser solubility of the salt at low temperature. At room temperature increasing the time beyond 15 minutes evidently has no further effect on the total penetration of the red and on the number of bands formed. The white on the other hand, had penetrated much farther at room temperature than in the cold.

As I see it, at room temperature, the formation of the white precipitate uses up most of the solute in gel so that just enough is left to form the red bands. At low temperatures the formation of the white proceeds slowly and uniformly and does not exhaust the solute so completely as at room temperature so that more solute reacts to form the red bands.

Macroscopic examination of these sections revealed that the bands of the cake at 35°C were thinner and therefore, appeared farther apart than in the room temperature cake. Also the bands at 35°C were broken while in the cakes kept at room temperature they were continuous.

VI. A study of the effect of gravity on the total penetration and on the formation of rings.

No work has been done as to what influence gravity has on this formation. The following experiment was therefore, taken up.

Glass tubing with the same inside diameter (9 mm.) was divided into two parts, each part 20 cm long. These were half filled with 5% gelatin containing 0.1% potassium chromate. the remainder of the tubes was filled with 1% silver nitrate and cork stoppers were inserted so that no air was left in the tube. One of the tubes was placed perpendicularly with the gelatin uppermost, the other with the gelatin down.

After one day both tubes had the same appearance with respect to red rings and white haze in advance. Immediately under the surface of the gelatin there were 6-7 fine red rings, barely visible to the eye. Following these were the customary coarse red rings.

After two days the inverted tube, i.e., that with the gelatin uppermost, showed a total penetration of 21 mm, 14 mm of which were occupied by the red rings and 7 mm by the haze, while in the normal tube, i.e., with the gelatin down, the total penetration was 25 mm, 18 of which were red and 7 mm were white.

After four days the difference was even greater although the general appearance and structure was the same in each tube. The inverted tube showed a penetration of 28 mm. and the normal tube showed 34 mm.

Conclusions. Gravity has no appreciable effect on the ring formation but where the entering ions are working against gravity as in the inverted tube, the total penetration is much less, showing that there is some effect due to gravity.

If we assume that the theory of Dhar and Chatterji is satisfactory then this result might be considered as substantiating it. For it seems reasonable to conclude that if the particles are peptized or colloidal as this theory claims, then the effect of gravity would be greater on these "large" particles than it would on individual ions or molecules.

VII. Liesegang's "dead space" experiment.

Holmes (2) cites Liesegang's (1) "dead space" experiment as confirmatory of his theory of the phenomena of periodic precipitation. Liesegang's experiment consisted of immersing a glass tube open at both ends filled with gelatin containing 10 per cent sodium chloride, in a solution of silver nitrate. The silver diffused in from both ends of the tube and precipitated as silver chloride at each end. However, these two precipitates did not meet in the middle of the tube. A clear dead space remaining in the center of the tube contained no sodium chloride at all because it had diffused away in opposite directions.

Not cognizant of this work I carried out a similar experiment with opposite results.

A glass tube open at both ends containing 0.1% potassium chromate in 5% gelatin was immersed horizontally in a silver nitrate solution. The silver ions entered the gelatin from both ends and precipitated in form of red rings preceeded by the white haze. The penetration and the number of rings formed were equal at each end. After about six or seven red rings had formed no further red deposit was laid down. The white precipitate grew considerably in advance of the last red rings from both ends, eventually meeting in the center.

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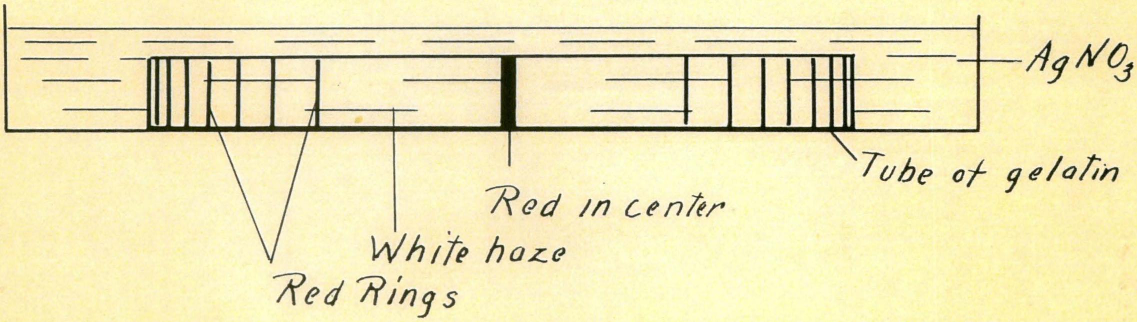
(1) Liesegand, Z. angew.Chem., 23, 2124, 1910.
(2) Holmes, Colloid Chemistry, p.806, edited by J.Alexander.

After several days a red precipitate also formed in the center.



It is very evident that all of the solute had not diffused from the center of the tube outwards as Holmes assumes.

Section III

In all of the preceding experiments where silver nitrate penetrated into a gelatin gel containing potassium chromate there has resulted two definite and distinct structures, the fine white hair lines (the substructure) and the coarse red bands or rings which are presumably silver chromate.

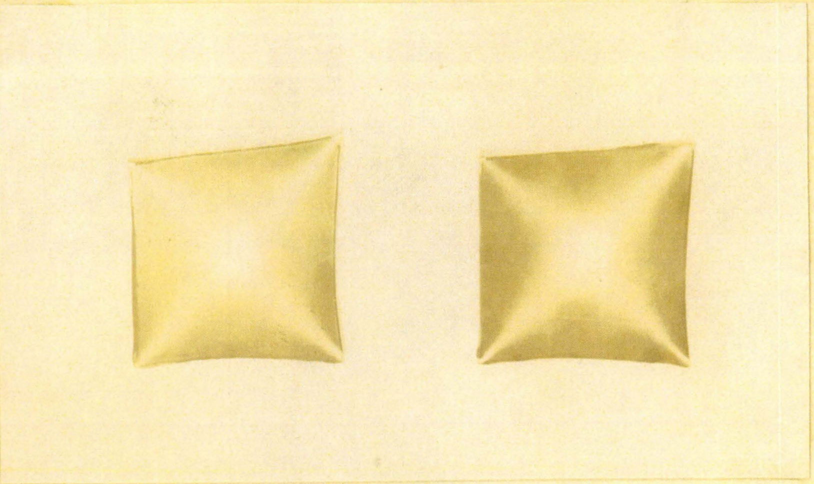
What is responsible for these white hair lines and what effect have they on the formation of the red bands?

From my own experimental work as well as from other sources I knew that this double structure is the exception and not the rule. No other combination of solutions gave such results. The conclusion arrived at therefore, is that there are two salts present in the gelatin, one which is added, the other which is present as an impurity in the gelatin.

The fact that the precipitate is white when the entering ion is silver and that on exposure to light the precipitate becomes dark in color, led me to think that the impurity was a chloride.

Test tubes containing ordinary 5% gelatin to which nothing was added were therefore made up and a 1% silver nitrate solution layered over the gelatin. In a short time there appeared at the surface of the gelatin the white precipitate which we have referred to as the white haze. The precipitate gradually grew down into the gelatin. Microscopic examination of this showed the fine white hair lines.

A cube of this same gelatin was immersed in 1% silver nitrate for 4 hours. The illustration is a photograph of a section taken through the center of this cube.



This photograph shows a well defined pattern, the corners of which are apparently clear. Microscopic examination, however, shows that the hair lines in the precipitate are continuous at the corners and that the precipitate is less dense here giving the section the appearance that the corners are clear. Evidently it is the impurity in the gelatin which has been responsible for this white fine lined sub-structure.

Liesegang(1) first noted that white fine hair lines formed in ordinary gelatin and ascribed them to sodium chloride: but considered that the fine lines had no influence on the formation of the red bands.

(1) Liesegang, "Ueber die Schictungen der Diffusionen (Engelmann, Liepzig, 1907); quoted from Kohler (see below); book not available.

F. Kohler (1) did extensive work in the study of the sub-structure, and decided that the fine lines were due to crystal of ammonium nitrate or potassium nitrate, the second product (by-product) of the reaction.

C.A. Schleussner (2) threw doubt on some of the evidence used by Kohler by indicating that the crystal shapes which play a part in Kohler's argument, were due to crystallization on drying the gelatin, and did not exist in the wet gelatin. Schleussner found that adding citric acid increases the distance between the red bands, but had no influence on the white lines; additions of citric acid and sodium chloride, both together and also separately, led him to the conclusion that the sodium chloride was essential to ring formation. He used a wabhed gelatin which, however, still contained halogens.

With the hope of obtaining a gelatin free from impurities samples from several different sources were examined. Included among these were the best gelatin used for culture media and Eastman's "ash free" gelatin. All of them, however, contained too much chloride impurity for our purpose.

It was therefore, decided upon to wash our granular gelatin with water to remove if possible, the chloride. For this purpose we used the following method taken from Loeb (3).

(1) F.Kohler, Koll.Zeit.,19, 65, 1916.
(2) C.A.Schleussner, Koll.Zeit., 31, 347, 1922.
(3) Jaques Loeb, Proteins and the Theory of Colloidal Behavior, page 35.

"The following method was used to prepare larger quantities of approximately isoelectric gelatin: 50 gm. of commercial powdered Cooper's gelatin, which happened to have a pH of 6.0 to 7.0, were put into 3,000 c.c. of M/128 acetic acid in a jar at 10°C., and stirred frequently. After 30 minutes the supernatant liquid was decanted and fresh M/128 acetic acid at 10°C. was added to equal the original volume. The mass was frequently stirred and after 30 minutes the acid was again decanted and replaced by an equal volume of distilled water at 5°C. The gelatin was well stirred and then filtered by suction through towel cloth in a Buchner funnel. It was then washed in the funnel five times each with 1,000 c.c. of water at 5°C. After all the water was drained off, the gelatin was transferred from the Buchner funnel into a large beaker which was then heated in a water bath to about 50°C. till the gelatin was melted. The concentration of the gelatin was determined by evaporating to dryness, using 10 c.c. of the melted gelatin in an electric oven at 90 to 100°C for 24 hours."

At a temperature of 5°C granular gelatin merely swells in water but does not dissolve. Following the washing of the gelatin and dissolving it in water instead of drying a portion at 90°C for 24 hours, I determined the concentration by evaporating a portion of the solution to constant weight at a temperature of 70°C for 48 hours. A higher temperature was avoided because some hydolysis might occur in the gelatin with prolonged heating.

The method of washing the gelatin with a dilute acetic acid solution was later abandoned because it was found that frequently some of the acid could not be washed out and this affected the results considerably, (see under influence of pH on the formation of rings).

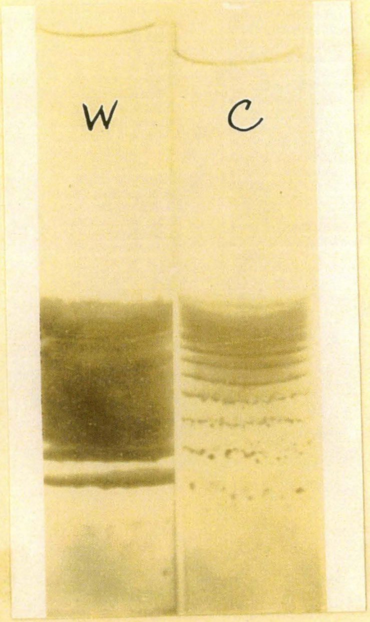
Instead the following method was used which gave us a pure gelatin usually very free from chlorides. Twelve to fifteen grams of granular gelatin were placed in a liter of distilled water at 5°C and left there with occasional stirring for 12 hours. When the gelatin had settled the water was poured off and a fresh supply added and left in contact with the gelatin for another 12 hours with occasional stirring. This was repeated two more times. The gelatin was then transfered to a large Buchner filter and freely washed with distilled water at 5°C for one hour. It was put in solution with distilled water and the concentration determined as before. The concentration was subsequently adjusted by adding a suitable amount of water.

I. First experiments comparing the penetration of silver nitrate into washed gelatin with ordinary gelatin both containing potassium dichromate.

A film of the washed gelatin (5) was allowed to set on a glass plate. A drop of 1% silver nitrate solution was placed on this film. Simultaneously a similar experiment was carried on with ordinary unwashed gelatin. After several hours the washed gelatin showed a continuous precipitate of red silver chromate with no evidence of rings or of any white haze. The ordinary gelatin on the other hand, showed the customary ring formation preceded by the white haze.

Using the same concentration of solutions the experiment was repeated using a test tube. After three hours the tube of unwashed gelatin showed 5 rings of red with the white cloud. The washed gelatin showed a solid red precipitate extending as far into the gel as the last ring of the other tube, with no white precipitate.

After one day the washed gelatin (W) still showed no rings while the unwashed gelatin (C) had 9 or 10 rings. After four days a band began to appear in tube W. The tubes after six days are shown in the following illustration.



In addition to the rings in C as shown in the illustration there are 4-5 smaller red rings near the top of the gelatin. The photograph was taken by transmitted light with the result that these rings at the top which were very close together appear as a solid structure.

- -

Silver nitrate was layered over some of this washed gelatin to which nothing was added. Just a bare trace of white precipitate could be detected. In the melted gelatin addition of silver nitrate produced no precipitate. Evidently the chloride impurity had been fairly well eliminated.

This same experiment of using potassium chromate in washed gelatin with silver nitrate over was repeated several times with other washed gelatins and in every case rings did not appear if at all, until after one or more days of reaction when only one or two rings would form while a control tube would form many red rings in a few hours. Moreover the appearance of these rings in the washed gelatin was entirely different from the unwashed. In the former they were coarser, heavier and more irregular suggesting a flat clot, while in the latter gelatin the rings were more delicate and finer.

As a result of the above work I felt satisfied that in the washing of the gelatin something, presumably the chloride, was removed which was necessary to the formation of the rings.

Could the rings be made to reappear in washed gelatin by the addition of a chloride?

Before this problem could be attacked it was necessary to know the approximate amount of chloride which was present in the ordinary gelatin so that the washed gelatin could be made up to that concentration of chlorine.

The following method of trial and error was resorted to in order to determine the approximate concentration of chlorides in the gelatin.

Washed gelatin (concentration adjusted to make a 5% solution) in 10 cc portions was poured into 7 special test tubes, selected for their uniformity of inside diameter. To each tube was added the following amounts of a 3% sodium chloride solution: #1 - 0.0cc; #2 - 0.025 cc; #3 - 0.05 cc; #4 - 0.1 cc; #5 - 0.3 cc; #6 - 0.6 cc; #7 - 1.0 cc. A similar tube containing 10 cc of a 5% solution of ordinary unwashed gelatin was also made up. After the gels had set 1% silver nitrate solution was layered over them in 5 cc portions to each tube at the same moment.

After several hours of reaction that tube of washed gelatin which showed the same amount, approximately, of precipitation, judged by the depth of penetration, as the tube of unwashed gelatin was selected as containing an equivalent amount of chlorides present in ordinary gelatin. The amount for the gelatin which we have used was found to be .01 grams as sodium chloride in 100 cc.

As a result of these conclusions we can attribute the failure of red precipitate to form as on page 39 because the silver chloride is more insoluble than the silver chromate and therefore precipitates before the chromate. There is not enough silver in the outside solution to satisfy both the chloride and the chromate and the chloride consequently uses it all.

II. Effect of the addition of sodium chloride to washed gelatin on the deposit of silver chromate.

Washed gelatin (5% solution) in a test tube (W_2) containing 0.1% potassium dichromate and to which was added sodium chloride so as to make the solution the same concentration of chloride as the unwashed gelatin, was covered with 1% silver nitrate. A control tube (C) of ordinary gelatin was made up to the same concentration of potassium dichromate and also covered with 1% silver nitrate.

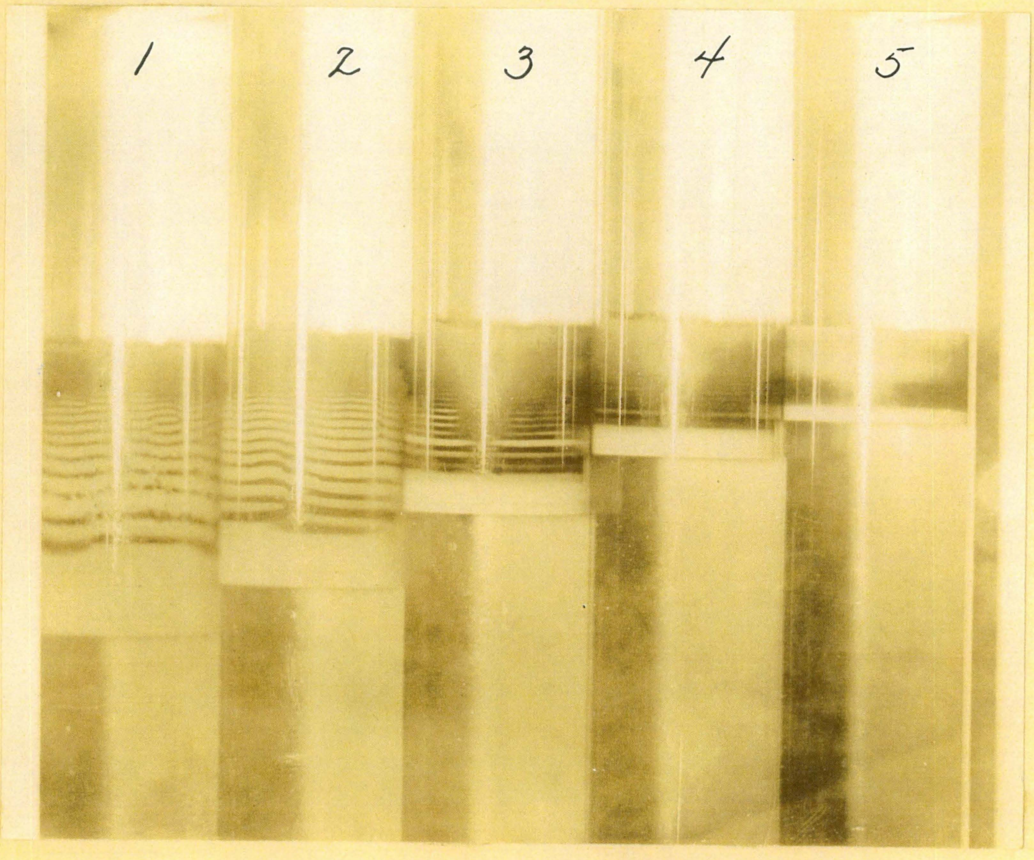
After three hours a dense red "shell" thicker than in the control (C) had formed in the washed gelatin (W_2) below which was one red band, the control showed 4 bands. A white cloud or haze preceded the red as in the control tube. After one day the tube (W_2) showed 4 rings as compared with 8 in tube (C). The rings were thicker and denser in W_2 than in C. After 3 days the tube showed 6 rings.

Evidently the presence of the chloride favors or is essential to the formation of the red rings although the formation was still different than in the control.

The question arose as to the possibility of error in the quantity of sodium chloride added. The following experiment was therefore undertaken.

III. The effect of varying the amounts of sodium chloride added to washed gelatin containing potassium dichromate. Also the effect on the total penetration of the silver ion.

Washed gelatin (5% solution) containing 0.1% potassium dichromate was added in 10 cc portions to five test tubes #1-5. Sodium chloride solution was added to each so as to make the concentration in 10 cc of gelatin as follows: #1 - 0.0015 grams; #2 - 0.003 grams; #3 - 0.009 grams; #4 - 0.018 grams; and #5 - 0.03 grams. The five gels were overlaid at the same moment with 5 cc of 1% silver nitrate.



The illustration shows these tubes after several hours of reaction. There is very good formation of rings in #1 and #2 which resemble very closely the formation in unwashed gelatin. Also the white haze which is very evident below the last ring is comparable to the haze in the unwashed gelatin. In #3 the rings are fewer and more compact while in #4 and #5 there are no rings.

It is very evident from these tubes and from others to which no chloride has been added that the formation of the red rings of silver chromate is dependent not only on the presence of the substructure caused by the chlorides but also on the concentration of the chlorides. Neither in a gelatin with no chloride impurity nor in gelatin containing the amounts of chlorides as in #4 and #5 is there any formation of rings. Between these two limits of concentration the rings of silver chromate form.

It is interesting to observe from this illustration that with increasing concentrations of sodium chloride the total penetration or depth of reaction decreases markedly. This seems somewhat paradoxical at first but can readily be understood if we consider that with a high concentration of the chloride in the gel there is sufficient chlorine present in the upper layers of the gelatin to react with all of the incoming silver. The greater the concentration of chlorine the less the silver will penetrate before being used up. Also as the chloride ions are removed by precipitation the osmotic pressure from below is great enough to cause a more rapid diffusion of the chloride ions into the upper zone of reaction. In other words, the diffusion gradient is greater the more concentrated the salt in the gel.

In the previous experiment I have assumed that it is the chloride ion which is responsible for the structure without paying any attention to the effect of the cation. It would be well to establish this point definitely.

Three tubes of washed gelatin 5% solution containing 0.1% potassium dichromate, 10 cc to each tube, were made up. The following chlorides were added in such quantities that the amount of chlorine would be the same in all tubes.
To #1 was added 0.00291 g of sodium chloride;
#2 0.00274 g of calcium chloride and to
#3 0.0037 g of potassium chloride. A control tube of unwashed gelatin was also made up. Silver nitrate (1.0% solution) was layered over the gelatin.

After 18 hours of reaction the tubes 1, 2 and 3 had the same appearance, each having 5 red rings with the same amount of white haze preceding the last ring. These tubes do not resemble the control in that they have a wide "shell" at the top while in the control the shell is narrow and the rings start close up to the top of the gelatin.

Since these tubes have the same structure we can conclude that it is the anion (Cl) which is responsible for the structure and that the cation has no effect.

IV. Effect of the previous addition of the second product (by-product) of the reaction.

Kohler attributes the fine line to the crystals of potassium nitrate (the second product of the reaction, see page 59). Recently Wo. Ostwald (1) stated that the previous addition of the reaction electrolyte changed the width and distance of the bands.

Using the same recipe as in the previous work, .005 grams of potassium nitrate were added to 10 cc of washed gelatin. Also .005 grams of potassium sulphate were added to another 10 cc. A tube of washed gelatin as a control was also used.

After 4 days of reaction the total penetration in all three tubes was the same, i.e., as solid red precipitate with no rings. During the next three days three rings had developed in all tubes. The rings were equally spaced and at equal depths. The control tube developed one more poorly defined ring after this which the others did not.

It is evident that in this case the previous addition of the by-product electrolyte had little if any effect on the ring structure. Also the effect of the potassium sulphate was negligible. I cannot agree with Kohler that the soluble reaction product is the cause of the fine lines.

(1) Ostwald, Kolloid Z. Special No. April 1, 1925, p.380-90.

Further work is necessary with various concentrations in order to check up on Ostwald's work.

In the pursuit of the previous experimental work in which test tubes were used frequently many tubes of gelatin had to be discarded because there appeared to be a dense precipitate between the gel and the glass. Evidently the gelatin in setting had not adhered to the wall so that when the liquid was poured on the gelatin it would leak down next to the glass wall and react with the surface of the gelatin. This was very unfortunate in that the precipitate was extensive enough to completely cloud the formation in the interior of the gel. Absence or presence of rings could not, therefore, be determined.

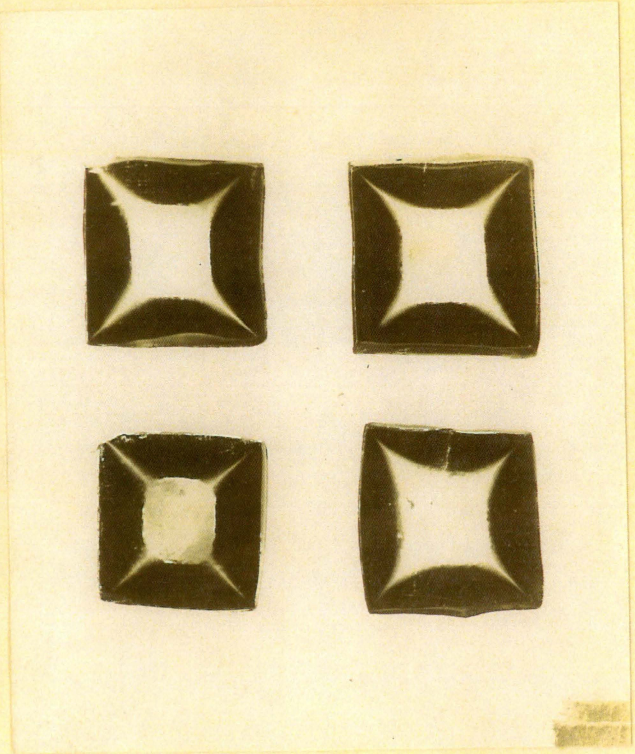
Also in using tubes, even the flat type, we were frequently unable to give the formation a good microscopic examination. The search for the existence of hair lines was particularly handicapped by the use of test tubes.

It was therefore, decided upon to permit the reaction to take place in cubes of gelatin where the entering ion would penetrate from all sides. A section taken through the center of such a cube, when mounted on a glass slide, would show the pattern formation. Microscopic examination with high magnification was possible by this method. The mounted section also lent itself better to photography than the tubes and the section when dried on the glass plate formed a permanent record.

The method of preparing such a cube of gelatin is as follows: The melted gelatin solution to which was added the required amounts of the salt was poured into a small 30 cc beaker and allowed to harden in the refrigerator. When sufficiently hard it was removed from the beaker and with a razor blade a cube 3 to 4 cm on edge was cut from this block of gelatin. The cube was then immersed in the outside solution for a definite period of time.

V. Pattern formation in a cube of washed gelatin.

A cube of washed gelatin containing 0.1% potassium dichromate was immersed in 1% silver nitrate for 4 hours at a temperature of 3°C. Sections 1 mm. thick taken horizontally through the center of the cube are shown in the illustration.

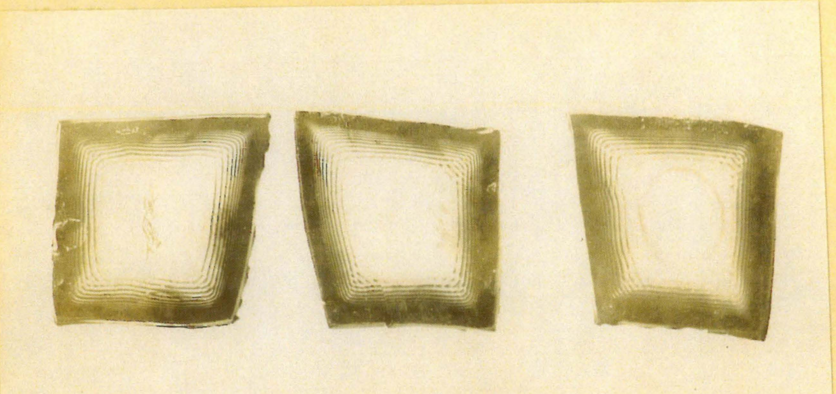


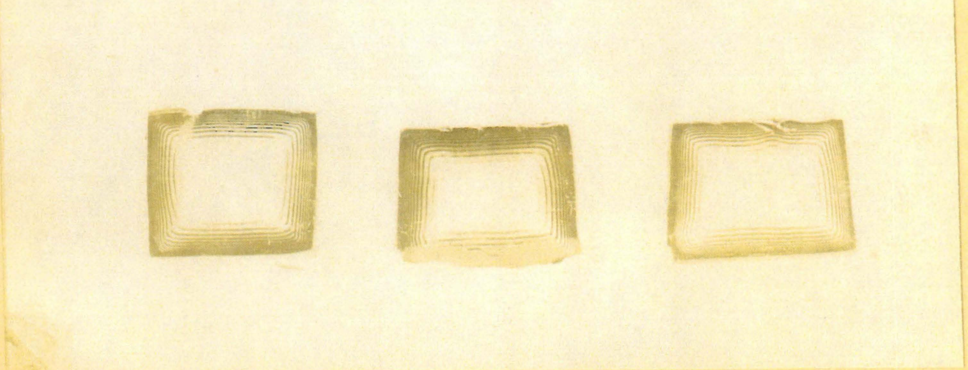
These sections show a well defined pattern similar to the lead chromate pattern. There is no evidence of any red bands or white haze. Also, unlike the picture of the cube on page 36 the corners are clear of any red precipitate. Microscopic examination failed to reveal any banded structure in the red precipitate and also any hair lines. Red particles could be distinguished.

This is further confirmation that in the absence of the hair line substructure the red banded structure failed to develop.

VI. Pattern formation in a cube of washed gelatin to which sodium chloride was added.

A cube of washed gelatin, made up the same as the preceding except that it contained sodium chloride in addition to the potassium ~~nitrate~~ dichromate, was immersed in 1% silver nitrate for 4 hours at 3°C. The following photographs of slices of this cake show that the red bands have reappeared.

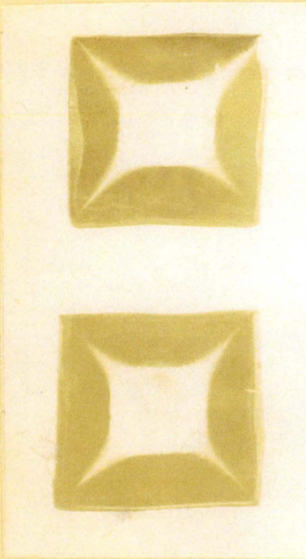




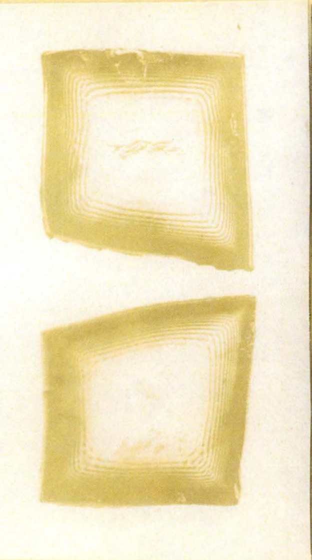
A white haze which is not apparent in the photograph is also present and in this a microscopic examination shows the presence of the fine white hair lines. In other words, the addition of the sodium chloride has caused a formation very similar to that of the unwashed gelatin.

The following shows (1) a section of the washed gelatin cube; (2) a section of the washed gelatin to which sodium chloride has been added and (3) a section of ordinary unwashed gelatin.

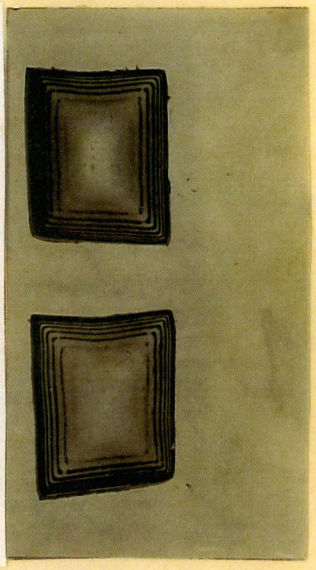
1



2



3



This experiment was repeated many times and a well defined pattern which was free from red bands was always obtained with the washed gelatin.

VII. Effect of H ion concentration of the solution on the ring formation.

It has been reported (1) that the effect of the gelatin in preventing the precipitation of silver chromate increases with increasing acidity. This should have some influence on the formation of the red bands but should not influence the white precipitate. In other words, the pH or H ion concentration of the gelatin may have a direct bearing on the formation of the red bands. It was decided to investigate this phase of the phenomena.

The pH of the gelatin solutions was determined by the colorimetric method; that is, the color of the gelatin and a certain amount of an indicator is compared with the color of a standard solution of known pH, equal color indicating equal pH. This method was not very accurate because the color of the gelatin must be overcome but for preliminary work it was suitable.

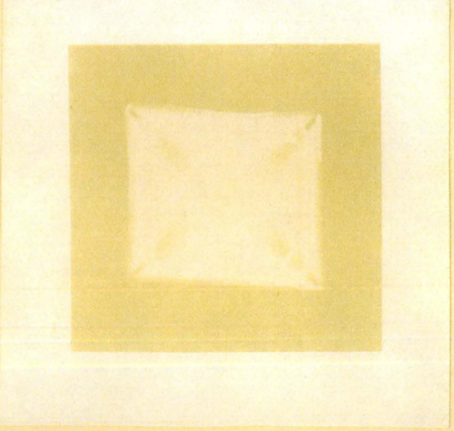
The pH determination was always made before adding the potassium dichromate because the yellow CrO_4 ion would screen the color of the indicator; also because the addition of potassium chromate increased the acidity beyond the range of my indicators.

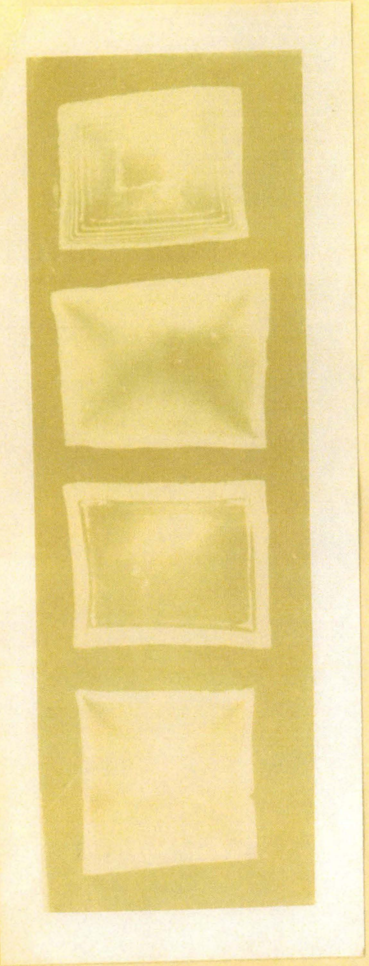
(1) Chemical Abs., 20, 2772, (1926).

Washed gelatin was adjusted to a pH 4 ; potassium dichromate added and the solution divided into two parts, 1 and 2. To #1 was added sodium chloride. Cubes were then made from both solutions and immersed separately in 1% silver nitrate for 4 hours at 3°C. Sections of both showed a very dense red precipitate penetrating about 1 mm. only into the gelatin. Section of #1 showed the customary white precipitate extending as usual to near the center of the cube, the white pattern is the same as that shown on page 58 . Section of #2 showed in addition to the red shell, some red spherical particles immediately below the shell. The remainder of the gelatin was clear. No red rings could be seen in either section.

At pH 4 therefore, the silver chloride pattern formed as usual but the silver chromate failed to precipitate beyond the outside edge. All of this is in conformation of work mentioned above.

Ordinary unwashed gelatin was adjusted to a pH of 4 and potassium dichromate added. A cube of this was immersed in silver nitrate (1%) for 4 hours with the result as shown in the illustration which is a print made by using the section as a negative.

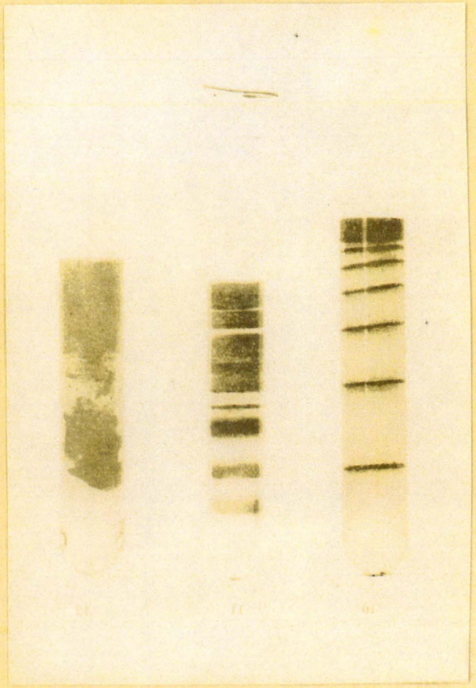




This also shows a failure of the silver chromate to form although there is evidence of two very poorly formed rings. The pattern due to the chloride impurity is very plainly shown.

VIII. Influence of low pH values on ring formation in test tubes.

Bradford (1) working with silver chromate precipitations in agar gels found that when citric acid was added to the gel, the formation of the silver chromate was changed considerably. The following illustration is taken from his article and shows the effect of increasing the amounts of citric acid.



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(1) Bradford, Biochemical Journal, vol. XIV.

#10 - N/150 K_2CrO_4; N/300 citric acid.
#11 - N/140 " ; N/250 " "
#12 - N/100 " ; N/140 " "

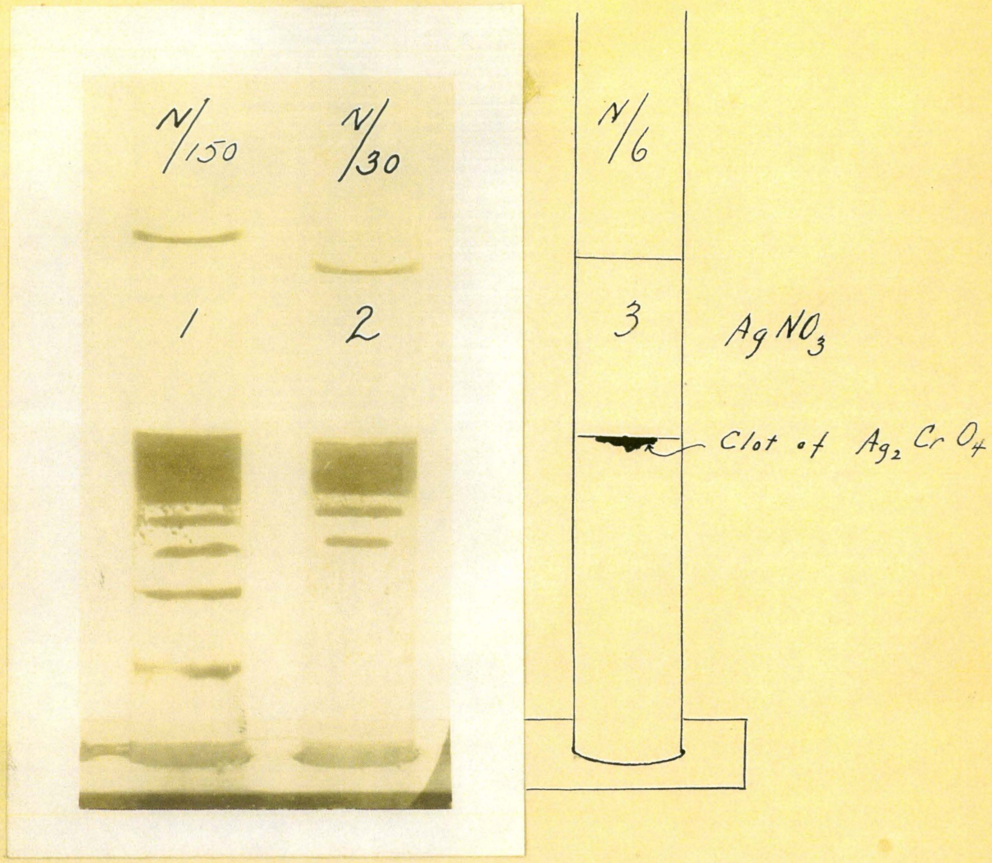
According to Bradford the effect of the citrate ion is to increase the supersaturation of the silver chromate before precipitation takes place.

Judging from my previous work on the effect of the H ion concentration I cannot agree with Bradford that it is the citrate ion which is responsible for the changes which he shows. I would rather attribute the effect to the H ion.

The following work was undertaken in order to study what effect increasing amounts of acetic acid would have on the formation.

Washed gelatin containing some sodium chloride and potassium dichromate was divided into three tubes. To #1 was added acetic to make the solution N/150. #2 was made N/30 and #3 was made N/6.

The gels were covered with 1% silver nitrate. The results after six days of reaction are shown in the illustration.



With increasing acidity there is a decreasing amount of silver chromate precipitate, and few rings are formed; tube #3 showing merely an irregular clot.

While my concentrations are greater than those which Bradford used, yet the results are in a general way a confirmation of his. His picture shows that a larger amount of acid as in #2 there is a tendency to clot formation similar to that which I had obtained in #3.

It is interesting to observe from the picture that in #2 the last ring is smaller than the preceding which also is smaller than the inside diameter of the glass tube.

IX. Effect of adding alkalie to the gelatin.

Ordinary unwashed gelatin containing 0.1% potassium chromate was poured into test tubes in portions of 10 cc to each tube. Sodium hydroxide, 1% solution, was added to each tube as follows:

#1 - 0.0 cc sodium hydroxide
#2 - 0.1 " " "
#3 - 0.5 " " "
#4 - 1.0 " " "

The gels after setting were covered with 1% silver nitrate solution.

After one hour #1 showed 6 red rings with a white haze; #2 also showed 6 rings with the white haze but the rings were finer than in #1; #3 showed very fine red lines barely visible to the eye below which was a solid red precipitate and the usual white haze. $3 also showed these fine rings at the top.

After three days of reaction #1 showed 10 rings with a structureless red precipitation below this; #2 showed more rings at greater depth than #1; #3 and 4 showed solid red precipitate below the fine rings mentioned above. The same amount of white haze containing hair lines preceded the red in all four tubes. In #3 and 4 the total penetration is less than in #1 and 2.

Conclusion: With increasing alkalinity the rings become finer and more numerous up to a certain alkalinity, beyond which rings do not form. The silver chromate is laid down in a solid red structure.

This conclusion received further confirmation as a result of work done with washed gelatin in form of cubes.

Sodium chloride was added to some washed gelatin containing potassium dichromate. The solution was divided into two parts #1 and #2. #1 was adjusted to a pH of 6 and #2 to a pH of 8. Previous work has shown that a variation of pH 6 to 7 caused no appreciable difference in the ring formation. The two cubes were immersed in 1% silver nitrate for 4 hours, sectioned and mounted.

Section of #1 (pH 6) had a shell about 1 mm thick and about 6 red rings. #2 (pH 8) had a shell about 3 times thicker than #1 with fewer rings inside of this. Both showed a white pattern with hair lines.

Section IV

Discussion.

Before considering further the theoretical aspects of the problem it would be well to review the various formations which are considered representative of the Liesegang phenomenon.

These formations may be divided into two classes, the typical and the atypical.

The typical formations are those in which the two components diffuse toward one another. The diffusion may occur in gas, liquid or solid (gel). Silver chromate is representative of the formation in gels, rings of it have been formed in gelatin, in agar and very recently Hatschek (1) formed rings of it in silicic acid gel, using very concentrated solutions of potassium dichromate (4%) and silver nitrate (25%).

Silver chloride rings have been formed in water by Bordorson (2). Wo.Ostwald (3) has also formed rings of several other substances in water. Koenig (4) formed periodic precipitates with gases. HCl gas diffusing one way and ammonia diffusing in opposite in tubing results in formation of bands and heaps of ammonium chloride. The bands form toward the gas which diffuses less rapidly. He also obtained bands with other gases.

I was able to obtain periodic precipitates by diffusing HCl gas against ammonia.

(1) Hatschek, C.A., 3112, 20, #19
(2) Broderson, Kolloid Z., 35, 21, 1924.
(3) Wo.Ostwald, Kolloid Z.,40, 144, 1927
(4) Koenig, J.Phys.Chem., 24, 473, 1920.

The atypical formations are:

Freezing water produces rings. Bechhold (1).

Gelatin layers on freezing form in rings. Rohonyi (2).

By evaporation of a solution of one part rabbit serum in 100 parts of physiological salt solution, rings of sodium chloride form which resemble the Liesegang phenomenon. Other concentrations of serum do not give it. De Nouy (3).

Von Weimarn (4) formed rings by evaporating a colloid solution of gold in a conical vessel. The rings formed on the walls.

Bechhold (5) found that diffusion of sodium chloride out of a solution of globulin in gelatin caused the globulin to be precipitated in turbid zones.

Handovsky and DuBois-Raymond (6) were able to obtain rings by diffusion of aklaloids (pilocarpine) into gelatin containing potassium iodide and iodine. Discoloration took place in forms of rings leaving the original colored gel between.

Fine suspensions on settling form sharply defined zones. Zacharias (7).

When colloidal mercury is added to blood agar plates very distinct Liesegang rings appear. Takogi (8).

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(1) Bechhold, Z.physik.Chem., 52, 185, 1905.
(2) Rohni, Biochem.Z., 53, 210, 1913.
(3) De Nouy, J.Exp.Med., 35, 707, 1922.
(4) Von Weimarn, Kolloid Z., 37, 78, 1925.
(5) Bechhold, Colloids in Biology and Medicine, by Bechhold, 1919.
(6) Handovsky & DuBois-Raymond, Kolloid Zeit., 33, 347, 1923.
(7) Zacharias, Kolloid Zeits., 34, 37, 1924.
(8) Takogi, Biochem.Z., 172, 483, 1926.

A consideration of these typical and atypical structures reveals that all of the leading theories which were presented in Section I of this paper were built up around the typical formations although the various authors did not draw any such distinction. The formation of atypical structures follows different laws and therefore any of the older theories could not be applied to them. Wo. Ostwald is the first to point out that his theory applies to only one class (the typical). Any theory in the future must also make this distinction.

Specific criticisms of these leading theories have been presented both in Section I and in the account of the experimental work. It would be well to criticize these theories collectively or in groups and endeavor to glean from them such facts, common to several, which could be used to support or disapprove one general theory which will be presented as a consequence of my own experimental work and the researches of others.

Briefly the theories may be tabulated as follows:

	Author	Key Note of Theory
1.	Ostwald	Supersaturation and metastable limits.
2.	Bechhold	Precipitate soluble in second product of reaction.
3.	Bradford	Adsorption of the solute in the gel by the precipitate.
4.	Holmes	Ionic activities and mass action law.
5.	Williams and MacKensie	Similar to Holmes'.
6.	Dhar and Chatterji	Peptization followed by coagulation.
7.	Wo. Ostwald	Diffusion waves and mass action law.

Bechhold's has been proven untenable, in fact he no longer advances it.

Ostwald, Holmes, Williams and MacKensie, and Dhar and Chatterji do not take into account the specific effect of the gel, although their theories assume that the reaction is occurring in a gel.

That a solid gel is unnecessary has been shown by many workers (see page 87) and that the nature of the gel has a specific effect cannot be denied for although we can obtain bands of silver chromate in three different gels yet the fact that the concentrations of the reactants necessary for the formation of rings vary so greatly for the different gels it is evident that the gel must have some specific influence.

Bradford's theory takes the influence of the gel into account and he has been able to produce rings in different gels by correcting for this influence. On the other hand, his theory assumes a solid gel.

Wo. Ostwald sums up the influence of the gel, metastable limits, peptization and coagulation, adsorption, etc., as not fundamental conditions for rhythmic banding but as secondary factors. His diffusion wave theory will need considerable experimental verification before it can be accepted. Furthermore, as mentioned previously, I am unable to understand the actual formation of a band from this theory.

The theories all agree on one point which is that the material from the gap region travels up to the layer of precipitation above and deposits there. The actual form of this material as to whether it is in true solution or as colloidal particles, solid or liquid, has been the subject of some controversy but in view of some recent work it is evident that this material is in colloidal form, the particles of which are sufficiently small to have an appreciable diffusion speed but which does not approach that of electrolytes.

If this material does ~~not~~ so exist then in the work of Lottermoser there is a clue as to the real cause for typical periodic structure.

The following is taken from his article (1).

"If 20 cc. of a N/10 solution of KI are diluted to about one liter and if to this solution, vigorously agitated by a motor driven stirrer, we add drop by drop a N/10 $AgNO_3$ solution from a burette, no clouding of the solution takes place after the addition of the first few drops. Instead the solution takes on a greenish color. On the further addition, drop by drop, of the $AgNO_3$ solution, the solution gradually becomes more and more opalescent, until finally, when nearly equivalent quantities of KI and $AgNO_3$ are present, there is observed a rapid increase in the opalescence. However, as long as KI is still in excess no real cloudiness can be observed. The solution still remains translucent when observed with an incandescent electric lamp behind it. Cloudiness followed by gathering together of a flocculent precipitate first takes place when KI and $AgNO_3$ are present in equivalent quantities in the solution. In the example just described a colloidal solution results as long as KI remains in excess. Complete flocculation of the AgI formed will occur when the KI and $AgNO_3$ are present in equivalent quantities.

(1) Lottermoser, from Colloid Chem., p.670 by J.Alexander.

Furthermore, it has been found that the colloidal formation is not limited to this particular case of an excess of KI. The reaction proceeds on the whole exactly in the same way when, for instance, 20 cc of a N/10 $AgNO_3$ solution which has been diluted to about one liter and which is vigorously stirred as in the foregoing experiment, has a N/10 KI solution added to it from a burette drop by drop. As long as there is an excess of $AgNO_3$ present the AgI remains in the colloidal state. As soon, however, as the $AgNO_3$ and KI are present in equivalent quantities complete flocculation of AgI takes place."

Adapting this work to the formation of rings in a gelatin we can say that the incoming silver ion joins with a chloride ion present in the gel, to form a colloidal silver chloride particle. So long as the chloride ions are in excess the silver chloride is formed as a colloid. Functioning as such these particles will not diffuse away as fast as the various electrolytes diffuse toward this zone of reaction. The chloride ions are continuously migrating upwards from immediately beneath this zone of colloidal silver chloride. The diffusion in this region, according to Fick's law is the greatest because here the concentration of chloride ions is the least. This process results in a poverty of chloride ions immediately below this zone of colloidal silver chloride. Meanwhile the silver ions are invading this zone and eventually exceed the chloride ions since their speed and number is greater because of the pressure behind them. According to Lottermoser, the silver chloride particles should now flocculate out. The gelatin merely serves to hold in place the precipitate as it forms. A liquid or gas in which there is no disturbance would act in the same manner. The silver ions continuing their advance into the gel meet but few chloride ions in the lean area below the precipitation and they must travel across this space before they will meet chloride ions in sufficient quantity to form enough colloidal particles of silver chloride so that when the silver ions are in excess a visible precipitate will result. This will repeat many times, colloidal silver chloride is first formed and then flocculated out forming a band, the Ag traverse the lean zone below this and again form the colloidal particles with the chloride ions.

Migration of the silver chloride particles is not necessary for this theory but neither is it denied by it for this theory admits of some movement of the particles but this movement is slower than that of ions. Neither does this theory deny that supersaturation, adsorption, peptization and coagulation and diffusion are unnecessary. These factors are, as Wo. Ostwald says, merely accessory.

This theory is not dependent on the form of solvent since any material in which the ions can diffuse and in which there are no disturbing influences such as convection currents the precipitate stays where it is formed.