Rhythmic Banding in Protoplasm

By

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Introduction

In the course of some work on the effects of heavy metals on protoplasm (Seifriz and Uraguchi, 1941), I had the opportunity to study striking pictures of rhythmic coagulation in slime mold protoplasm.

Rhythmic zone formation or periodic precipitation in protoplasm and in tissues has been observed by a number of investigators. Lloyd (1928) has reviewed the subject. Lloyd and Moravek (1928) have compared the artificially produced banded precipitates in trichomes with those in gelatin in capillaries. An extensive study of rhythmic zone formation in plant tissues has been made by Küster (1913 and 1931). Balbach (1936) observed an alternation of granular plasma and hyaloplasm encircling particles of platinum black sprinkled on the plasmodia of slime mold. Weide (1939) saw similar zone formation in phycomycete plasmodia. Seifriz (1939) regards the formation of strata in slime mold plasmodia as a type of cytoplasmic coagulation, bearing a very obvious resemblance to Liesegang bands.

Observations

The material used in this work was the plasmodium of the slime mold, Physarum polycephalum. Stock cultures were kept growing on moist filter paper, and fed powdered oatmeal. For experimental study, small portions of a plasmodium were transferred to cover slips which had been coated with thin films of agar. These subcultures were kept in a moist chamber for several hours, time enough for the protoplasm to spread on the surface of the agar. The small plasmodia thus prepared were bathed in the following salt solutions, or the solutions were injected into the plasmodia by means of micropipettes.

The precipitation of rhythmic zones was observed on the application of all of the following solutions to slime molds, but in the case of none among numerous others tried. No buffer mixture, hydroxide, or acid at a pH above 3 produced rhythmic precipitates.
On bathing transferred plasmodia in the effective solutions, rhythmic zones begin to appear in scattered areas. There is first formed a densely granular center surrounded by a narrow hyaline, or less granular zone (Figs. 1, 2, 5, and 8). There then appears a dark granular band followed by alternating zones of hyaline and granular protoplasm extending centrifugally.

<table>
<thead>
<tr>
<th>Salts</th>
<th>pH</th>
<th>Concentrations in molarity</th>
</tr>
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<tbody>
<tr>
<td>Cu(NO₃)₂</td>
<td>above 4.5</td>
<td>0.01, 0.0005</td>
</tr>
<tr>
<td>Cd(NO₃)₂</td>
<td></td>
<td>0.1, 0.05, 0.01, 0.0005</td>
</tr>
<tr>
<td>Ba(NO₃)₂</td>
<td></td>
<td>0.1, 0.05</td>
</tr>
<tr>
<td>Fe(NO₃)₃</td>
<td>below 3</td>
<td>0.05</td>
</tr>
<tr>
<td>UO₂(NO₃)₂</td>
<td></td>
<td>0.1, 0.05</td>
</tr>
<tr>
<td>Hg(NO₃)₂</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acids</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO₃</td>
<td>1.4, 1.8, 2.2</td>
</tr>
<tr>
<td>HCl</td>
<td>1.4, 2.0</td>
</tr>
<tr>
<td>Buffer</td>
<td>2.8, 2.4</td>
</tr>
</tbody>
</table>

Fig. 1. Plasmodium bathed in dilute nitric acid: (pH 1.4). (×110). Fig. 2. Plasmodium bathed in 0.1 molar Cd(NO₃)₂. Fig. 3. Plasmodium bathed in 0.0005 molar Cu(NO₃)₂. (×730). Fig. 4. Plasmodium bathed in 0.1 molar Cd(NO₃)₂. (The coagulated core of the system has been washed away during handling.) (×110).

Quite a number of banded “islands” are formed within a plasmodium (Fig. 1). As each island enlarges the active streaming protoplasm is pushed back. The protoplasm within the banded areas is coagulated. The borderline between coagulated plasma and normal streaming plasma is usually clearly marked (Figs. 6, 9, and 11).
The rapidity with which alternating bands are formed, and their breadth vary considerably, depending on the nature of the solution, its concentration, and on the condition of the plasmodium. Thus, the zones formed in Cd(NO₃)₂ solutions of 0.1 to 0.01 molarity are much broader than those in any other solution.

After death caused by any of the foregoing salts, or acids, a plasmodium may be covered with ring systems (Fig. 1). Occasionally, two centers independently started became enclosed by a common system (Figs. 1, at left, and 3).

In some instances, as in Cd(NO₃)₂ solutions of 0.1 molar and 0.05 molar concentration, two neighboring granular bands separate after the completion of the ring system and in so doing stretch the intervening hyaline zone which then tears (Figs. 2, 4, and 5) forming thin thread-like strands between the two granular zones (Figs. 2 and 5).

Fig. 5. Plasmodium bathed in 0.05 molar Cd(NO₃)₂. (×110). Fig. 6. Plasmodium injected with 0.1 molar UO₂(NO₃)₂. Fig. 7. Plasmodium injected with 0.05 molar UO₂(NO₃)₂. Fig. 8. 0.05 molar UO₂(NO₃)₂ applied externally with a micropipette. Fig. 9. 0.01 molar Cu(NO₃)₂ applied externally with a micropipette.

The foregoing phenomena all take place in plasmodia bathed in solutions. Similar zone formation occurs when salts are injected into plasmodia with the aid of a micropipette mechanically controlled in a micromanipulator (Seifriz 1936). When a salt solution is injected into a plasmodium, the protoplasm surrounding the tip of
the pipette coagulates immediately. Several layers of concentric rings then appear centrifugally around the dark coagulum (Fig. 6). Occasionally when a solution is injected, further concentric bands are not formed after the initial precipitation of a coagulum around the tip of the pipette, followed by a single narrow hyaline zone (Fig. 7), which ends the process. It would, therefore, appear that there are conditions other than specific salts and acids of definite concentrations for the production of concentric bands. This has been shown to be true for the formation of Liesegang rings in gelatine (Küster 1931, and Lloyd and Moravek 1928 and 1931). What these conditions are cannot be said. They are included in the “physiological state” of protoplasm. In non-living systems temperature is a factor.

When banded precipitation is produced by the injection of solutions, the alternating zones are formed only at the point of injection (Fig. 6), whereas in bathing many scattered centers appear, all forming concentric rings simultaneously (Fig. 1). The widespread formation of many separate islands of banded precipitates can be prevented even when the solution is externally applied if instead of complete immersion, the salt is concentrated at a restricted local area by means of a pipette. Such an application results in a single, superficial, semicircular banded precipitate (Figs. 8 and 9).

Discussion

Certain salts and acids applied to slime mold plasmodia, either by immersion or injection, react with the proteins of the protoplasm in such a way as to produce periodic precipitation which results in the formation of concentric rings around the center of diffusion. The hydrogen ion and the ions of heavy metals bring about this banded precipitation of the proteins in protoplasm. In the case of salts, such as Fe(NO₃)₃, Hg(NO₃)₂, and UO₂(NO₃)₂, which greatly lower the pH of their solutions, the H⁺ ion is probably as much responsible as the metal ion, if not more so. In the case of Cd(NO₃)₂, Cu(NO₃)₂, and Pb(NO₃)₂, the pH of each of which is above 4.5, it is undoubtedly the metal alone which is responsible.

The banded precipitation of the proteins in protoplasm by acids and heavy metals is an example of the Liesegang phenomenon. The method of formation and the general appearance of the final picture are the same in the living material as in a non-living gel; thus, the breadth of the hyaline zones usually increases, and the degree of aggregation of the granules, or depth of color of the granulated
zones decreases, as the distance from the center increases (Fig. 10), which is characteristic of Liesegang phenomena.

The presence of scattered islands of banded precipitates surrounded by living and actively streaming protoplasm suggests that the protoplasmic surface is not uniform in composition or structure. When a plasmodium is immersed, the surrounding solution is in full contact with the entire surface, yet the dissolved substances enter only at certain isolated points around which the concentric bands are rhythmically precipitated. The permeability and composition of the membrane are therefore not uniform.

The precipitation patterns are in the main rather uniform as to type. However, sometimes the symmetry of the pattern differs considerably from the usual. Fig. 11 shows one of the irregular formations where a net structure has been formed.

Other irregularities occur which, however, are true to type but appear irregular because they are formed in restricted areas. When rhythmic precipitates are formed in strands, space limitations prevent development of the complete pattern (Figs. 10 and 12).

The periodic precipitations reported by Lloyd and Moravek (1928) in which the reagents meet in a gelatin layer formed in the capillary space between two glass plates, are closely similar to the banded patterns formed in slime mold plasmodia when bathed in the salts of heavy metals or in acids.

**Summary**

1. The bathing of the protoplasm of slime molds in various salts and acids, or the injection of these into protoplasm, brings about
the formation of rhythmic bands through the precipitation of protoplasmic proteins.

2. Both the hydrogen ion and the ions of heavy metals produce banded precipitates in protoplasm.

3. The periodic precipitation of concentric rings in protoplasm bears a striking resemblance to the Liesegang phenomenon.

4. The more rapid entrance of solutes at certain restricted and isolated points of the plasmodium indicates pronounced differentiation in the permeability of the surface.

Acknowledgment

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Literature Cited


