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# Chemotaxis in the Cellular Slime Molds

## I. The Effect of Temperature

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## INTRODUCTION

The aggregation of myxamoebae in the Acrasiae, or cellular slime molds, has long been regarded as a response to chemotactic agents (Olive, 1902; Potts, 1902). Runyon (1942) discovered that the factor responsible for the formation of centers and the inflowing of streams of myxamoebae could act through a dialysis membrane. Bonner (1947) provided convincing evidence that this was a chemical substance, which he termed acrasin. The attracting compound is heat stable and is destroyed by a protein of high molecular weight, probably an enzyme (Shaffer, 1953, 1956; Sussman *et al.*, 1956).

Only scant evidence of a temperature effect on aggregation is available in the literature. Potts (1902) observed a delay in the onset of aggregation if the myxamoebae of *Dictyostelium mucoroides* were incubated at a low temperature. Raper (1940) transferred cells of *Dictyostelium discoideum* to a higher temperature and found that aggregates were formed earlier than in control cultures. The resulting pseudoplasmodia were smaller in size.

The objective of this study was to determine the effect of different temperatures on cell aggregation; attention was paid specifically to the influence of temperature on chemotaxis in *Dictyostelium discoideum*. Small populations of washed myxamoebae were deposited upon a specially prepared agar (Konijn and Raper, 1961) on which the cells normally remained within the confines of the droplet. The cells moved outside its boundary only if attracted by acrasin that was secreted by aggregating myxamoebae in a neighboring population. The distance over which sensitive myxamoebae were attracted was

measured at various temperatures. The observed attraction was correlated with the time interval between the onset of aggregation and the time when almost all cells had entered the center of the aggregate in the attracting populations.

## MATERIALS AND METHODS

Myxamoebae of the haploid strain *Dictyostelium discoideum* NC-4(H) were grown in darkness in association with *Escherichia coli* No. 281 on a glucose–peptone medium (Bonner, 1947). The cells were harvested in the preaggregative stage and suspended in a 100 × diluted Bonner's salt solution (Bonner, 1947). The myxamoebae were centrifuged 2 or 3 times to remove excess bacteria. After resuspension, the cells were divided into two aliquots: one for the attracting population and the other for the responding population. Drops deposited directly after centrifugation were called attracting populations, because aggregations formed within these populations attracted myxamoebae of the later-deposited responding populations, causing the latter cells to move outside the boundaries of their drops. The aliquot that was reserved to provide the responding populations was resuspended in a full strength salt solution and stored at ca. 5°C until used as described below. The other cell suspension was adjusted to ca. $2 \times 10^7$ cells per milliliter and kept in cold 100 × diluted Bonner's salt solution. Small drops of this latter suspension were deposited with a handmade micropipette on plates of highly purified agar previously stored at ca. 5°C. The rigidity of this highly purified agar was kept low to allow attraction of cells outside the boundaries of the responding populations; only 30–35 gm of weight were required to push the end of a microscope slide into the agar (Konijn and Raper, 1961). This hydrophobic agar normally does not allow movement of cells outside the boundaries of the small populations. A harmful hypotonicity of the agar was prevented by restoring salts to the equivalent of Bonner's solution.

Each drop contained 2000–4000 cells and had a diameter of 0.5–0.7 mm after implantation. About 160 drops were deposited on the agar surface of each petri dish. The dishes were wrapped in aluminum foil to keep the cells in darkness and to allow a rapid adjustment of the temperature inside the dish to that of the incubator. For a temperature difference of 10°C (19–28.5°C) the adjustment to within 2°C from the required temperature took place within ca. 30 minutes. The

dishes were incubated at 8°C, 13°C, 18°C, 24°C, and 28.5°C in different compartments of a "Holima" gradient incubator. The temperature in each compartment varied ±1°C.

To provide responding populations, droplets of myxamoebae in the full strength salt solution were deposited 3–7 hours later. Because populations incubated at lower temperatures aggregated later, the time interval between implantation of the attracting and responding drops was increased at lower temperature. This delay in deposition was an attempt to get a similar physiological age in the various responding populations at the time they would be attracted by aggregations in neighboring drops. The drops were deposited at predetermined distances from the attracting populations varying from 100 $\mu$ to ca. 1500 $\mu$. Drop size and cell density were similar to those of the attracting populations. The agar was sufficiently weak to permit myxamoebae of the responding populations to move outside their margins if these cells were attracted by aggregations in neighboring populations (Konijn and Raper, 1966). The attraction was observed after streams of myxamoebae had entered the aggregates in the attracting populations and before the cells in the responding populations had formed their own aggregates. The distance between the center of the aggregate in each attracting population and the nearer margin of the responding drop was measured at a magnification of 80× through the ocular micrometer of a phase contrast microscope. Cell attraction over this measured distance was marked positive if myxamoebae moved outside the margins of the responding drop toward the attracting source, and negative if they stayed within the boundaries of these drops. The distance over which cells in 50% of the responding populations moved outside their drop margins toward the attracting populations was taken as a criterion of the attractive power of the aggregating myxamoebae. Test plates were exposed hourly to light for a count of the number of aggregations that were formed. Control plates were kept in continuous darkness until the distance over which attraction took place had to be measured.

## RESULTS

The chemotaxis was less effective when cells were incubated at a high temperature than at a low temperature (Fig. 1). At 28.5°C cells in 50% of the responding populations crawled outside the margins of

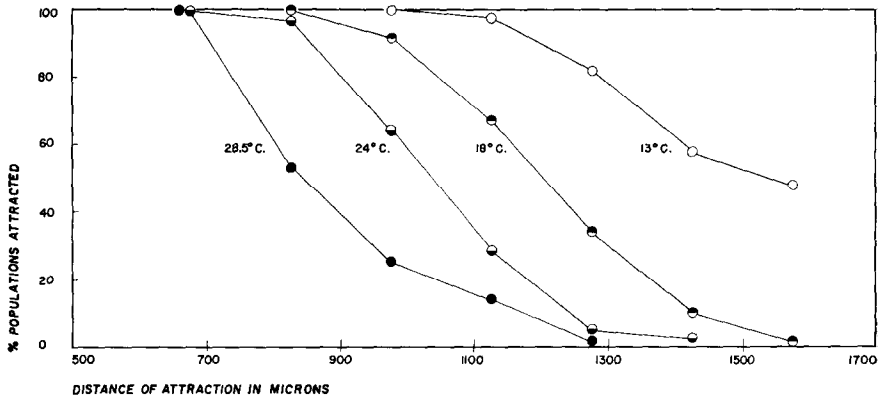


FIG. 1. The percentages of myxamoeba populations that show a response to developing aggregations under different temperature conditions and the distances between the nearest margins of these populations and the aggregations that attract them. Results represent the average of three experiments. ○ 13°C; ◓ 18°C; ◒ 24°C; ● 28.5°C.

their drops if the distance between the centers of the aggregates and the closest side of the responding populations was 850 $\mu$. At 24°C this distance increased to 1000 $\mu$, at 18°C to 1200 $\mu$, and at 13°C to 1550 $\mu$.

The onset of aggregation was delayed at low temperature. Raper (1940) had noticed this before under different cultural conditions. At 28.5°C 50% of the attracting populations started to aggregate after 4–6 hours. This time interval between deposition of the cells and the onset of aggregation increased to 8–11 hours at 13°C. At still lower temperatures responding cells were attracted over even greater distances, but aggregation was much delayed. At 8°C, for example, cells came together in irregular patterns but no definite centers were formed within 25 hours. At this low temperature cells of neighboring drops could be attracted even before developing aggregates could be clearly observed in the attracting populations. Also at room temperature, attracting populations could induce a response in a neighboring drop before the beginning of visible aggregation.

Myxamoebae of small populations are attracted to the centers in pulses, these being easily demonstrated by time lapse photography at intervals of 10 seconds. If the attracting and responding populations were observed in the same field, pulses in the attracting population were seen to be transmitted to the responding population, which may be as far as 500 $\mu$ from the attracting drop. Pulsations in

both populations appeared to be synchronized. Pulses in one large population could already be observed before the emergence of compact centers, as also has been shown in films made by Arndt and Bonner. These pulses were in all likelihood induced by acrasin, secreted by cells that occupy the center of pulsation. Since these early pulses were transmitted through the agar and elicited synchronized responses in the neighboring drops, the chemotactic substance appeared to be secreted before cells actually had formed definitive centers.

Plates exposed hourly to light showed the same, or slightly less, attraction than the plates that were kept in constant darkness. Only at 28.5°C were the myxamoebae in the exposed plates attracted over slightly larger distances than in the control plates.

Figure 1 does not indicate whether the effect of temperature on the attraction of sensitive myxamoebae was exerted during aggregation or in the preaggregative phase. Plates containing test populations were incubated at an higher or a lower temperature shortly before aggregation, or when aggregates were already developing. At 19°C, 50% of the attracting populations had started to aggregate 6 hours after deposition of the washed cells. At 28.5°C, aggregates were formed after 4 hours (Fig. 2). In small populations that were transferred from 19°C to 28.5°C after 4 hours, myxamoebae aggregated 1 hour later. If transfer from 19°C to 28.5°C took place 6 or 7 hours

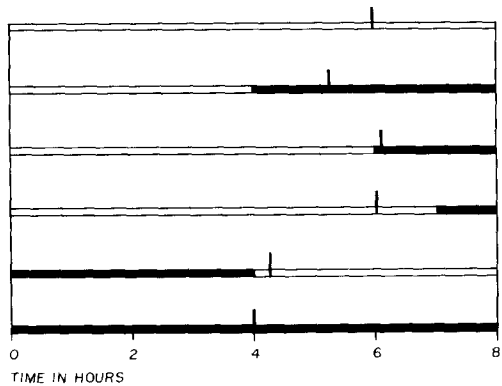


FIG. 2. The effect of an increase or a decrease in temperature on the time interval between the deposition of the myxamoebae and the beginning of aggregation. The graph represents three experiments. ——— 19°C; ▬▬ 28.5°C; vertical bar, time at which 50% of the attracting populations started to aggregate.

after deposition of the myxamoebae, the time of aggregation was not affected since aggregation was then near at hand, or had already begun. The change from 28.5°C to 19°C occurred after several populations had already started to aggregate.

The chemotactic response at 19°C was over a greater distance than at 28.5°C. Attracting populations which were transferred from 19°C to 28.5°C after 4 hours aggregated slightly more than 5 hours after their deposition. The attraction exerted on the responding populations was similar to the chemotactic response in populations that were kept constantly at 28.5°C (Fig. 3). When the transfer to 28.5°C took

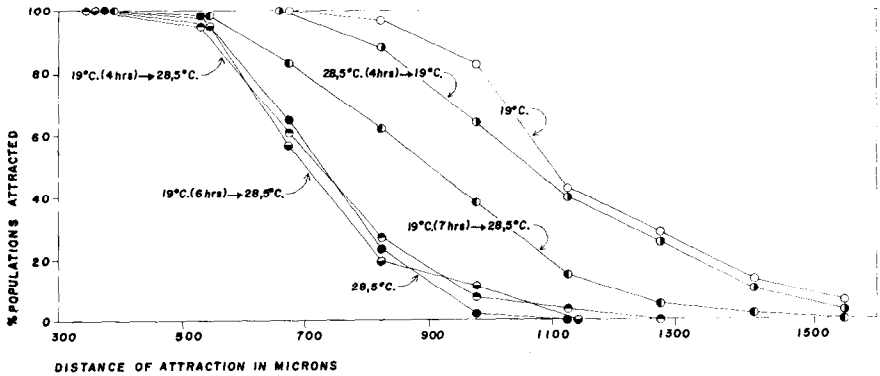


FIG. 3. The percentages of myxamoebae populations that show a response to developing aggregations under different temperature conditions and the distances between the nearest margins of these populations and the aggregations that attract them. Results represent the average of three experiments. ○ 19°C; ◓ 19°C, after 4 hours at 28.5°C; ◒ 19°C, after 6 hours at 28.5°C; ◐ 19°C, after 7 hours at 28.5°C; ◑ 28.5°C, after 4 hours at 19°C; ● 28.5°C.

place after 50% of the attracting populations had already begun to aggregate, attraction was still exerted over the same distance as in plates that were incubated at 28.5°C for the entire time. Exposure to higher temperature after 7 hours, when most of the populations had started to aggregate, resulted in an attraction over larger distances than in dishes that were kept at 28.5°C constantly (Fig. 3). The decrease in slope suggests that the first aggregates exerted their influence at a temperature more effective for attraction than aggregates that were formed later. If the attracting populations were incubated at 28.5°C, and transferred at the beginning of aggregation to 19°C,

the distance over which attraction was observed was only slightly less than in populations that were kept constantly at 19°C. These results indicate that the distance over which attraction occurs is not predetermined during the preaggregative phase. The temperature appears to affect the chemotactic response at the time the myxamoebae in the attracting drops are coming together. The variation in attraction in different experiments, caused by differences in cultural and environmental conditions, is under investigation.

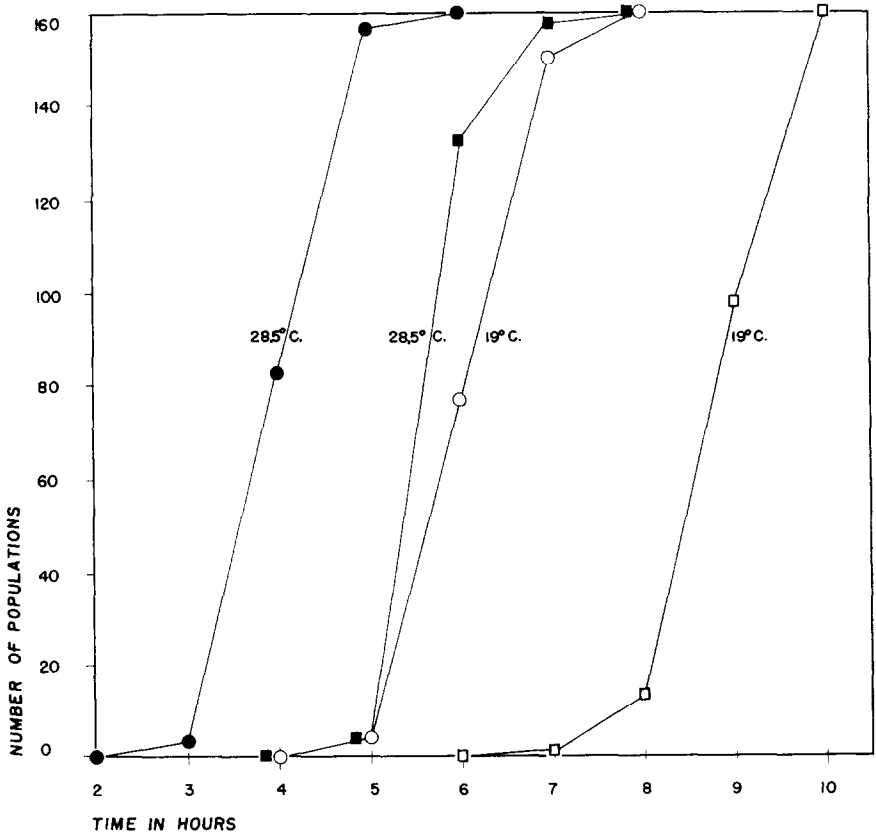


FIG. 4. The time interval between the beginning of aggregation (○ ●) and its completion (□ ■) and the number of populations that started or finished aggregation. Results represent the average of three experiments. ○, □: populations are incubated at 19°C; ●, ■: populations are incubated at 28.5°C.

The question remains whether temperature affects the attraction directly, e.g., by inactivation of acrasin, or indirectly, e.g., by extending the time over which cells in the responding drops are attracted. The latter possibility was investigated by a comparison of the attraction at different temperatures with the time interval over which the chemotactic agent was secreted. Responding cells are attracted mainly from the moment the cells in the attracting populations come together to form a definite center until the streams have entered the aggregate. Hourly observations were made to count the number of populations that had started to aggregate and the populations in which the streams of myxamoebae had entered the center (Fig. 4).

TABLE 1

RELATION BETWEEN THE DISTANCE OVER WHICH THE RESPONDING POPULATIONS ARE ATTRACTED AND THE TIME REQUIRED FROM THE BEGINNING OF AGGREGATION UNTIL ITS COMPLETION AT VARIOUS TEMPERATURES

| Temperature (°C) | 50% attraction ($\mu$) | Aggregation time (hours) |
|---|---|---|
| 13° (Fig. 1) | 1535 | 4.2 |
| 18° (Fig. 1) | 1195 | 3.3 |
| 24° (Fig. 1) | 955 | 3.0 |
| 28.5° (Fig. 1) | 863 | 2.0 |
| 19° (Fig. 3) | 1158 | 2.8 |
| 28.5° (4 hrs)—19° (Fig. 3) | 1096 | 2.3 |
| 19° (4 hrs)—28.5° (Fig. 3) | 716 | 1.8 |
| 28.5° (Fig. 3) | 717 | 1.7 |

The time interval between the moment at which 50% of the attracting populations started to aggregate and at which 50% finished aggregation was used as a measure of the duration of aggregation. This interval was 1.7 hours at 28.5°C and 2.8 hours at 19°C. The time period required from the onset till the end of aggregation was also measured at other incubation temperatures; the experiments are presented in Figs. 1 and 3. The resulting time intervals and corresponding distances of attraction are given in Table 1. Although large variations occur between the different experiments, the increased period of time necessary for aggregation at lower temperatures is obvious. This increase was correlated with an increase in the distance of attraction.

## DISCUSSION

An incubation temperature of 20–24°C has been considered optimal for growing cultures of *Dictyostelium discoideum* (Raper, 1940). Data presented in this paper show that an optimal temperature for culturing this species does not coincide with an optimal temperature for maximal attraction of sensitive responding cells or with the optimal temperature for early aggregation. At relatively high temperatures, which still allow the myxamoebae to aggregate, the time interval between deposition of washed cells and their subsequent aggregation is minimal. The distance over which sensitive myxamoebae are attracted also is minimal at high temperatures. When Raper (1940) found that cultures grown at 20°–24°C formed aggregates earlier if transferred to 28°–30°C, the enhanced aggregation was probably not due to an increased effectiveness of the chemotaxis. The chemotactic response appeared to be more pronounced at lower temperatures, with a maximal attraction at 11°–13°C. Under the applied conditions cells formed irregular patterns at 8°C. If a system could be developed which allows normal aggregation at 8°C, or lower, the distance over which attraction occurs may be still greater.

At low temperatures, cells of neighboring responding populations were attracted before the beginning of aggregation could be observed. At room temperature attracting populations seemed to affect the cells in the responding populations only after a center of aggregation had begun to develop. Time lapse films of an attracting and a responding population in the same field showed, however, that also at room temperature cells in the responding populations are influenced by the attracting population before any compact center of aggregation is formed. Some chemotactic substance, or acrasin, is apparently secreted some time before myxamoebae aggregate.

The optimal light effect for early aggregation in *Dictyostelium discoideum* was obtained by exposing cells to light in the preaggregative phase a few hours before the beginning of aggregation (Konijn and Raper, 1965). The attraction of myxamoebae appears not to be influenced by the prevailing temperature during the preaggregative period. Only at the time when the myxamoebae in the attracting populations are aggregating was the chemotactic response of neighboring cells affected by temperature. The chronological age of the attracting myxamoebae did not affect the distance over which the chemotactic response was noticeable.

The reduced attraction at higher temperatures was probably not due to a lack of sensitivity, for the responding populations that were incubated at 24°C and 28.5°C aggregated shortly after the last streams had converged to form compact centers of aggregation in the attracting drops. The diffusion rate of acrasin is not responsible for the reduced attraction at high temperatures, since we may expect that the increased temperature would favor diffusion. Shaffer (1956) demonstrated that an active acrasin solution lost nearly all its effect after storage at room temperature for 20 minutes. A solution thawed in the cold was still very active. Higher temperatures may therefore be responsible for an increased inactivation of acrasin.

Another factor that could favor an increased chemotactic response is an extended period of time over which acrasin is secreted. The time interval between the onset of aggregation and its completion was markedly longer at low temperatures. This extended period over which aggregation takes place suggests that the increased attraction can be at least partly explained by the longer exposure to chemotactic substances at low temperatures. A lower temperature would then not be the direct cause of an increased attraction, but its effect would be more indirect by extending the period over which the cells in the attracting populations complete their aggregations.

## SUMMARY

The effect of temperature on chemotaxis in the cellular slime mold *Dictyostelium discoideum* has been studied by incubating small populations of washed myxamoebae at different temperatures. Droplets containing a cell suspension of known density were deposited on a hydrophobic agar surface. The myxamoebae normally stayed within the boundaries of the implanted droplets, but they moved outside the margins of such droplets when they were attracted by acrasin secreted by neighboring populations. Sensitive cells in the responding populations were mainly attracted between the beginning of aggregation and its completion in the attracting populations. Secretion of acrasin before the formation of compact centers of aggregation was demonstrated at low temperatures and with time lapse photography.

A decrease in temperature was correlated with: (1) an increase in the distance of attraction; (2) an increase in the time interval between the beginning of aggregation and its completion; and (3) an increase in the lapse of time between the deposition of the myx-

amoebae and the beginning of aggregation. The chronological age of the attracting cells did not seem to affect the chemotactic response. The increased attraction at lower temperatures was correlated with a longer period between early aggregation and its completion. Only the temperature during aggregation had an effect on the distance over which cells could be oriented; any temperature change applied during the preaggregative stages was of no consequence to the attraction at later stages.

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