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pH affects fruiting and slug orientation in *Dictyostelium discoideum*

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SUMMARY

We have demonstrated two interesting facts about the transition from the migration stage to the final fruiting stage of *Dictyostelium discoideum*. One is that fruiting is favoured on acid substrata, and secondly, migrating slugs tend to migrate towards the acid side of a pH gradient. The suggestion is offered that these results can be interpreted in terms of the effects of NH_3. It appears to be an additional mechanism (besides phototaxis and thermotaxis) to assure that the final fruiting takes place in a favourable environment.

INTRODUCTION

There has been a concern for some time with the question of what factors influence migrating slugs of *Dictyostelium discoideum* to turn into fruiting bodies. Raper (1940) reported that light, a rise in temperature, and a decrease in humidity all favoured early fruiting. Later we suggested that humidity played an especially significant role (Bonner & Shaw, 1957) although this interpretation of those experiments is doubtful in the light of recent work. Next, Newell, Telser & Sussman (1969) reported that overhead light stimulated fruiting and that there was a factor given off by the slugs that enhanced migration. Schindler & Sussman (1977) discovered that this chemical stimulus for migration was NH_3 and that the absence of NH_3 encouraged fruiting. More recently, we examined the effect of overhead light and found that the light lifted the slug off the agar by phototaxis, and that this positional change of the slug promoted fruiting (Bonner *et al.* 1982).

Here we present experiments which show that the pH of the substratum not only affects the transition to fruiting, but migrating slugs are oriented by pH gradients. Earlier, Raper (1939) reported that if drops of HCl were placed near colonies of amoebae, the more HCl in the drop, the greater the number of fruiting bodies. Furthermore, he observed that the slugs migrated towards acid regions in the Petri dish. We have tried, unsuccessfully over the years to repeat this experiment, but never could exactly duplicate the required conditions. Now, using a totally different method, we have confirmed Raper's original observations. Furthermore, we suggest an hypothesis that brings together the role of light and NH_3 in the stimulation of fruiting *D. discoideum*

Keywords: pH, gradient, *Dictyostelium*, slug orientation, fruiting.

MATERIALS AND METHODS

Dictyostelium discoideum (NC-4) was grown with *Escherichia coli* (B/r) at 21 °C on nutrient agar. The amoebae were washed by centrifugation and concentrated in masses on non-nutrient agar (see Bonner *et al.* 1982, for details) to obtain large migrating slugs. The first set of experiments were run at 21 °C, and the pH gradient experiments were done at 12 °C, the latter temperature being especially favourable for prolonged slug migration.

Fruiting body formation and pH

A series of five experiments were run in which developing amoebae were composed on sets of Petri plates containing agar at two different pH concentrations. Half of the plates (at both pH's) were placed in the dark, while the other half were placed in a chamber with a source of light under the agar to attract the tips toward the agar surface so that the slugs would tend to hug the agar (see Bonner *et al.* 1982, for details). At different time intervals (plating the amoebae = time 0), the plates were removed and scored for percent fruiting (*vs.* percent migrating slugs). This was done by placing the plates over a grid divided into 0·5 mm squares and they were examined under a dissecting microscope at a magnification of × 25. The number of slugs and fruiting bodies for a series of grid squares were scored involving a total of 100–400 pseudoplasmodia for each experimental condition. In all but one case, there were duplicate plates which were scored separately.

In the first two experiments, $NaHP0_4/KH_2P0_4$ buffer was used at 10^{-2} M. The initial pH in one set of plates was that of the buffer mixture: the more alkaline set of plates was produced by adding different amounts of 1N-NaOH. Agar was then added to the buffer, autoclaved and poured into Petri plates (15 × 85 mm, plastic). The pH was determined before adding the agar, after the plates

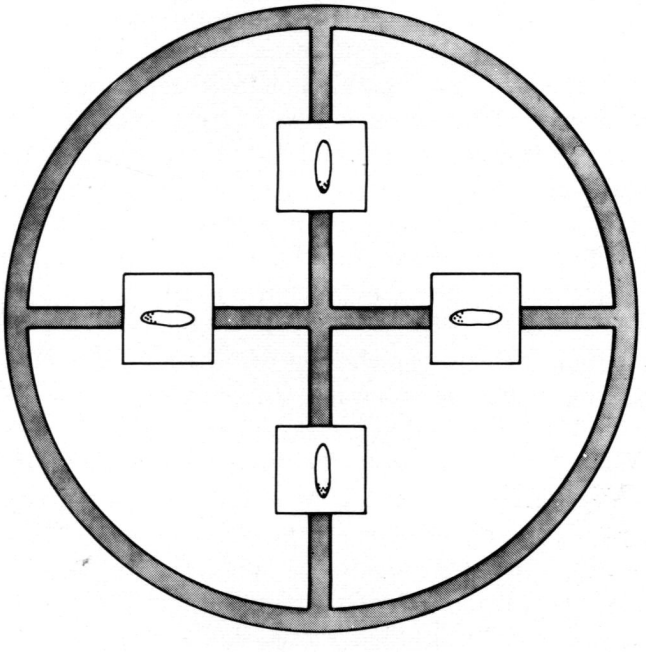

Fig. 1 A diagram (not drawn to scale) showing the placement of the slugs in the compartmentalized Petri dishes.

had cooled, and after the experiment was over; in each case the values held reasonably steady, even when the pH was at the extreme range of the buffer. In the next three experiments, a $NaHP0_4$/citric acid (Sorensen's) buffer was used at $16{\cdot}7 \times 10^{-3}$M. Care was taken to see that the total concentration of solutes in the agar did not vary significantly since it has been stated previously that an increase in the solutes results in a higher percent fruiting of bodies formed in a given time interval (Slifkin & Bonner, 1952). In the first two experiments, the paired pH's were 6·3–8·9, and 6·0–12·0. In the next three, they were replicates at pH 6·7–8·0.

The final experiments were run in Felsen-type Petri dishes in which the agar is in four separate quadrants or compartments (Falcon X plastic Petri dishes–15 ÷ 100 mm). Agar of one pH is put in two opposing quadrants, and the agar of another pH is put in the intervening quadrants. Each quadrant is separated from its neighbour by a plastic ridge. As before, slugs were formed from centrifuge-washed amoebae, placed on 2% agar. Slugs that had separated out from their neighbours were removed by cutting the 2% agar under them in a block approximately 15 mm square. These were then placed on the ridges of the compartments with the orientation shown in Fig. 1. In this way, the agar block straddled the plastic ridge and touched agar of two different pH's on each side. The experiments were run at 12 °C in the dark.

Three different buffer systems were used: MES for pH 5·5, 6·0 and 6·5; HEPES for pH 7·0, 7·5 and 8·0; and TRIS for pH 5·5, 6·0, 7·5 and 8·0, all at 15×10^{-3}M. Controls were run for all these buffers at the stated pH's in which all four compartments contained the same buffer. Again the pH's were tested before and after the experiment, and the deviation was found to be negligible. After 2 h and 4 h, the slugs were scored for their direction of migration.

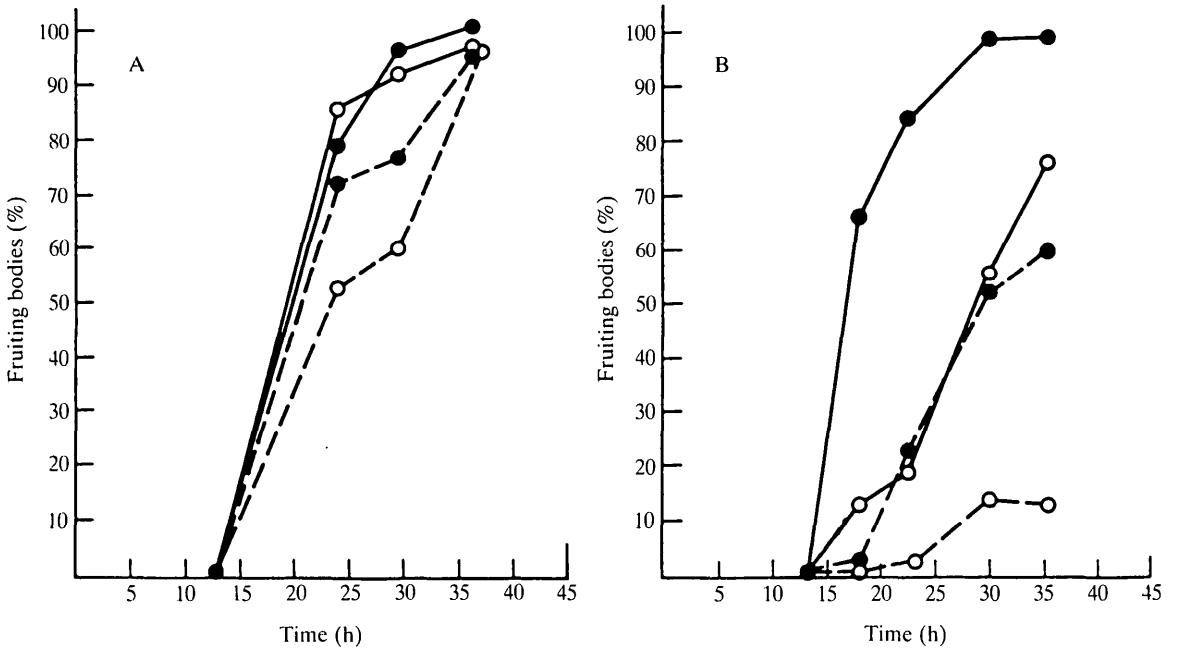

Fig. 2 The increase in the percentage of fruiting bodies over time under different conditions of light and pH. Open circles = light below; solid circles = dark. (A) pH 6·7 (solid line) compared with pH 8·0 (dashed line). (B) pH 6·0 (solid line) compared with pH 12 (dashed line). Given enough time all the slugs turned into normal fruiting bodies under all conditions.

RESULTS

The effect of pH on the rate of fruiting body formation

In all the experiments, both in the dark and with the light below, the fruiting bodies formed more rapidly in the more acid or low pH plates. This can be clearly seen in one of the three pH 6·7–8·0 experiments (Fig. 2A) and is very striking in the pH 6·0–12·0 experiment (Fig. 2B).

If the time to achieve 50 % fruiting body formation is averaged in the four experiments in which the pH difference is approximately 2·0 units, in the higher pH plates it takes 2 h longer in total darkness, and roughly 4 h longer in the plates with light below. The effect is even more obvious in the fifth experiment where the pH difference is 6 units (Fig. 2B). Therefore, a combination of high pH and light below

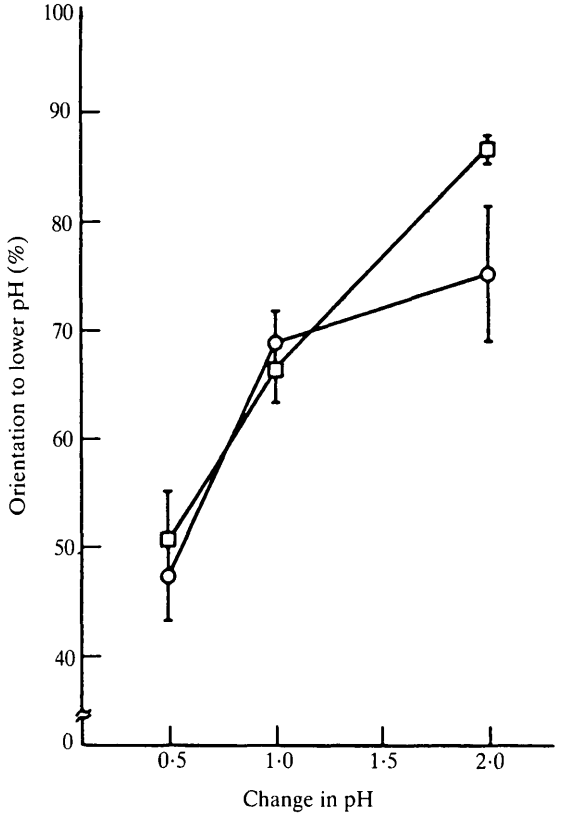

Fig. 3 The percent of slugs orienting towards the more acid side of a gradient plotted against the steepness of the pH gradient. Circles = 2 h; squares = 4 h recordings. (Data from seven experiments involving 448 slugs. The slugs that remained unoriented or twinned were not included.) For the pH differences of 0·5 and 1·0 all three buffers, MES, HEPES, and TRIS gave essentially the same result and were therefore combined. For a pH difference of 2·0 TRIS was used at pH 5·5–7·5 and 6·0–8·0, again with similar results and combined.

(which causes the slug tips to point into the agar surface) is especially effective in prolonging migration.

Evidence for migration slug chemotaxis in pH gradients

We have a total of seven experiments in which migrating slugs were placed in different pH gradients. When all the data from these experiments were combined, it was obvious that the steeper the pH gradient, the greater the orientation towards the acid side (Fig. 3). If the difference in pH's in the quadrants was 2·0 units, then by 4 h, 87 % of the slugs were oriented towards the lower pH. This was true for both ends of the pH scale tested. Orientation in all the control plates was random (in 11 experiments, involving 558 slugs).

DISCUSSION

In a previous study (Bonner *et al.* 1982) it was shown that the reason overhead light induces fruiting in migrating slugs, as Newell *et al.* (1969) had discovered, was that the light lifted the tip of the slug from the agar surface by phototaxis and this lifting process led to fruiting. In view of the results described here, we can ask what might be the molecular stimulus that causes aerial tips to lead to the final differentiation of spores and stalk cells, and the formation of a fruiting body.

Initially we postulated that it might have something to do with surface tension within the water film and if the slug tip is in the film, migration continues. This hypothesis was put forward on the presumption that the whole reason for fruiting is to break through the surface film and push the spore mass up into the air. Using a non-toxic detergent (Tween 20) we were able to lower the surface tension of the water film significantly, but this in no way affected the rate of fruiting (data not shown).

Our results presented here showing that low pH favours fruiting leads us to suggest that the overhead light effect on fruiting could best be explained in terms of ammonia relations. Schindler & Sussman (1977) and Sussman & Schindler (1978) demonstrated that an increase in ammonia caused a prolongation of migration. If we ask what effect pH has on ammonia, the higher the pH the greater the NH_3/NH_4^+ ratio. Our results in which alkaline agar favours migration and acid agar favours fruiting are totally consistent with the Schindler and Sussman observations; relatively more NH_3 favours migration.

Now we must account for the fact that when the light is below, as compared to total darkness, migration is prolonged even further. It is possible to imagine two ways in which NH_3 influences fruiting: by the overall amount of NH_3 due to the effect of pH, and by the amount of NH_3 lost from the surface of the slug by diffusion. Slugs in the dark will be oriented at random, many of them rising into the air. Those slugs surrounded by air will lose NH_3 far more rapidly than those which cling to the water film on the surface of the agar. If this hypothesis is correct, then the reason overhead light promotes fruiting is that it allows NH_3 to diffuse rapidly away from the free slugs that rise from the agar surface by phototaxis.

Let us consider the intriguing fact that slugs seem to migrate towards a low pH environment that favours fruiting. This chemotactic response of whole slugs is weak by comparison to their phototactic and thermotactic response, and, as one can see from Fig. 3, a relatively steep pH gradient is needed in order to produce a significant effect. If the pH difference between the two quadrants of the Petri dish is 2·0 then one can assume that over a distance of about 3 mm, the gradient would involve a 100-fold difference in hydrogen ions. We have only tried fair-sized slugs, so assume that the width of the slug is 0·3 mm, then this will produce a difference of 10-fold in H^+ between the sides of a slugs. It is quite possible that it is not the H^+ gradient that is significant, but the gradient of NH_3. This raises the further interesting possibility that NH_3 might be the repellant gas involved in the orientation of rising pseudoplasmodia described previously (Bonner & Dodd, 1962).

Finally we can ask whether or not this pH chemotaxis might have some ecological significance. There is a common thread in all migrating slug taxes: they bring the slugs from regions of the soil where fruiting is less desirable to where it would be more so. And the presumed reason for this is that positions near the surface of soil are more effective for spore dispersal, while deeper regions are more suited to the earlier feeding stage. This would explain why the slugs go towards light. It would also explain the extraordinary sensitivity to temperature gradients (Bonner, Clarke, Neeley & Slifkin, 1950; Poff & Skokut, 1977), especially since recently Whitaker & Poff (1980) have shown that at cool temperatures migrating slugs are negatively thermotactic, while at warmer temperatures they are positively chemotactic, and the range of these two effects is determined by the temperature at which the amoebae grow. This could be explained by the fact that at night the soil surface would be colder than below (hence negative thermotaxis of the slugs if they want to move towards the surface), while in the daytime the reverse is true, and again this would lead to the slugs going upward. We can now add to this list of speculations the possibility that migrating slugs can move away from regions high in NH_3 which would be characteristic of deep chambers in the soil laden with bacterial food, to regions relatively free of NH_3, such as the more aerated soil surface. If there is any truth to these ideas, it is remarkable that so many different mechanisms evolved to insure spore masses being placed optimally for dispersal.

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