THE EFFECT OF SALTS AND ORGANIC SOLUTES ON THE MIGRATION TIME OF THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM [1]

MIRIAM K. SLIFKIN AND JOHN TYLER BONNER

University of Illinois, Urbana, Illinois, and Dept. of Biology, Princeton University, Princeton, New Jersey

As Raper (1935) showed when he first described the amoeboid slime mold *Dictyostelium discoideum,* this particular species is unique in that it possesses a migration stage in its life cycle. The independent, separate amoebae of the vegetative stage stream together to form cell masses or pseudoplasmodia, and these pseudoplasmodia migrate over the surface of the substratum until the final culmination stage. In culmination there is both a rising into the air and a cell differentiation; the result is a fruiting body in which a delicate stalk made up of large vacuolate amoebae supports an apical mass of encapsulated amoebae or spores.

The duration of this migration period has always been known to be extremely variable, from a matter of a few hours to approximately two days, and Raper (1940) demonstrated that low temperature and high humidity favored prolonged migration to some extent. While these observations have been confirmed we have also found that the concentration of solutes in the medium proves to be a far more important limiting factor, and by varying this environmental condition it has been possible to control and to greatly extend the duration of migration.

Methods

The organisms were cultivated on standard medium (Bonner, 1947)[2] with *Escherichia coli* at room temperature for two days, at which time the amoebae had usually begun to aggregate. Plugs of the standard agar covered with the aggregating amoebae were cut out with a glass ring 10 mm. in diameter and placed on a circular coverslip of equal diameter, which was then set on the medium to be tested. The coverslip was used so that no diffusion could take place between the plug of standard medium and the test medium. All the test plates were incubated at 17° C. The technique thus far is essentially similar to that used by Bonner *et al.* (1950).

After 24 hours a sufficient number of migrating pseudoplasmodia had moved on to the test medium so that the plug and coverslip could be removed. Each of the migrating pseudoplasmodia that remained was marked by placing a drop of ink on the bottom of the petri dish at 24-hour intervals until fruiting occurred. In cases where the pseudoplasmodia split longitudinally all the parts were followed.

[1] The preliminary experiments of this study were carried out at Princeton University with the help of a grant from the American Cancer Society.

[2] There is an error in the formula of the culture medium in Bonner (1947) and KH_2PO_4 should be substituted for the incorrect K_2HPO_4.

The test media were prepared by adding the different solute concentrations to 2% Difco Bacto-Agar and each solute was tested separately. All solutes were chemically pure.

Results

First a test was made using the standard medium. As can be seen from the table, in 137 cases the mean migration time was 2.0 days (± a standard deviation of 1.3) and the range extended from 1 to 5 days. This is in keeping with the previous reports in the literature, but if the migrating pseudoplasmodia were allowed to migrate in 2% agar alone, the mean time was extended to 10.3 days (see table) and in one instance the migration lasted 20 days. As a matter of fact the mean value is in this instance of dubious significance because 29% of the pseudoplasmodia disappeared completely, presumably from a combination of loss of cells by straggling in the posterior track and the loss of protoplasm used in the energy of locomotion. The smaller the original size of the pseudoplasmodium, the sooner it would disappear, but there was no evident correlation between duration of migration before fruiting, and the size of the pseudoplasmodium.

As can be seen from the table and from Figure 1, if any solute was added to the 2% agar, the greater the concentration of the solute the shorter the mean duration of migration. This was true for the three organic non-electrolytes tried (dextrose, sucrose, dl-alanine) and the three electrolytes (NaCl, Na_2HPO_4, $CaCl_2$). None of the non-electrolytes was significantly different from any other, but as a group the salts were from 4 to 6 times more effective at a given concentration. Among the salts, NaCl and $CaCl_2$ were very similar in their effectiveness, while Na_2HPO_4 was twice as effective.

If concentrations of solutes higher than those given in the table were used, only a few pseudoplasmodia crawled off the standard medium plug even though they readily left plugs from the same standard plate when put on media of lower concentrations. There was one exception to this: in the case of the 6% dextrose a good number of pseudoplasmodia migrated off the plug, but as soon as they touched the surrounding medium they fruited. This happened in 78 cases without any deviations and represents a condition of minimum migration. It was also noticed on repeated occasions that prolonged migration never occurred over the surface of glass, but that if the pseudoplasmodium reached the side of the petri dish, it fruited shortly thereafter.

Discussion

The cessation of migration and the onset of fruiting is a major step in the differentiation process of *Dictyostelium*. It is of interest therefore to observe that the concentration of the solutes can play a part in the initiation of differentiation. Considering the importance of concentration, the effect would appear to be an osmotic phenomenon, but this would neither account for the more effective nature of the salts, nor the fact that the salts differ among themselves when their effective osmotic pressure is calculated. In the future it is hoped that by testing other substances it will be possible to understand more thoroughly the mechanism of initiation of fruiting and the basis for the variability of effectiveness of different substances.

There is a parallel to the phenomena found here in the work of Smith and Grenan (1949), who have found that the concentration of the medium will affect

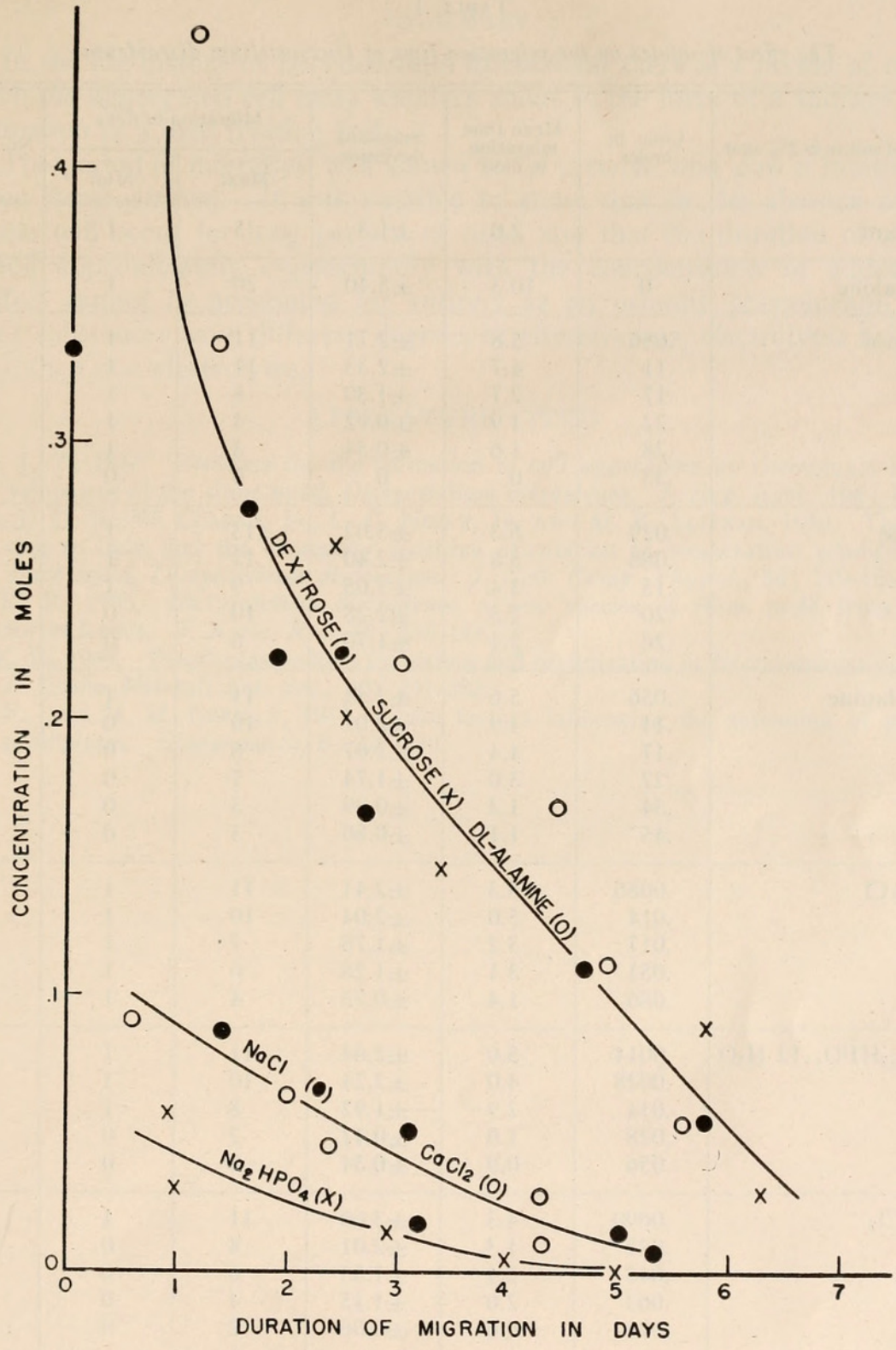

FIGURE 1. A graph showing the relationship between the concentration of solute (moles) in a 2% agar test medium and the duration of migration of the slime mold *Dictyostelium discoideum* in days.

the degree of spreading of the plasmodium in the true Myxomycete, *Physarum polycephalum*. Again with increasing concentrations there is a decrease in spreading, which suggests the possibility that in *Dictyostelium* the solutes affect the motility of the cell mass and therefore indirectly initiate final differentiation.

TABLE I

The effect of solutes on the migration time of Dictyostelium discoideum

% Weight of solute in 2% agar	Conc. in moles	Mean time migration in days	Standard deviation	Migration in days		No. of cases
				Max.	Min.	
Std. medium		2.0	±1.3	5	1	137
2% Agar alone	0	10.3	±5.40	20	1	142
1% dextrose	.056	5.8	±2.71	11	1	79
2%	.11	4.7	±2.33	11	1	53
3%	.17	2.7	±1.30	8	1	154
4%	.22	1.9	±0.92	4	1	56
5%	.28	1.6	±0.54	3	1	53
6%	.33	0	0	0	0	78
1% sucrose	.029	6.3	±3.02	13	1	120
3%	.088	5.8	±2.40	12	1	59
5%	.15	3.4	±2.05	9	1	58
7%	.20	2.5	±2.28	10	0	29
9%	.26	2.4	±1.70	6	0	33
0.5% dl-alanine	.056	5.6	±2.34	11	1	82
1%	.11	4.9	±2.02	10	0	94
1.5%	.17	4.4	±2.07	8	0	64
2%	.22	3.0	±1.74	7	0	59
3%	.34	1.3	±0.99	3	0	43
4%	.45	1.1	±0.80	3	0	17
0.05% NaCl	.0086	5.3	±2.41	11	1	73
0.08%	.014	5.0	±2.04	10	1	76
0.1%	.017	3.2	±1.76	7	1	90
0.3%	.051	3.1	±1.28	6	1	71
0.5%	.086	1.4	±0.75	4	1	74
0.05% $Na_2HPO_4 \cdot 12\ H_2O$	.0014	5.0	±2.64	11	1	109
0.1%	.0028	4.0	±2.24	10	1	49
0.5%	.014	2.9	±1.92	8	1	141
1%	.028	1.0	±0.42	2	0	113
2%	.056	0.9	±0.54	2	0	37
0.1% $CaCl_2$	.0090	4.3	±2.60	11	1	63
0.3%	.027	4.3	±2.01	8	0	63
0.5%	.045	2.4	±1.53	6	0	47
0.7%	.063	2.0	±1.15	4	0	26
1.0%	.090	0.6	±0.06	2	0	24

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SUMMARY

1. In the life history of *Dictyostelium discoideum* there is a period of migration in which the aggregated cell mass wanders about in the form of a sausage prior to the formation of a final fruiting body.

2. The period of migration was known to be variable, and now a limiting factor has been demonstrated. It was possible to show that in the absence of solutes migration will occur for long periods of time, and that the duration of migration decreased approximately exponentially with the concentration of added solute. The effect cannot be accounted for entirely as an osmotic phenomenon because different substances have different degrees of effectiveness, electrolytes being more effective than non-electrolytes.

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