Excystment of the Amoeba *Hartmannella castellanii*

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The germination of protozoan cysts is an important stage in the life cycles of those organisms which form them. Despite the existence of pure cultures of many soil amoebae and the development of the replacement technique for studying their encystment under relatively controlled conditions (Band, 1964; Neff, Ray, Benton & Wilborn 1964; Griffiths & Hughes, 1969) previous studies of excystment have not been carried out under axenic conditions. This communication describes investigations of the excystment in bacteria-free conditions of cysts of *Hartmannella castellanii* which were produced by the replacement technique.

The growth and encystment of *Hartmannella castellanii* have been described previously (Griffiths & Hughes, 1968, 1969). Cysts produced by the replacement technique were stored as suspensions in distilled water at 4°. The cysts were collected by centrifuging at 2000 g for 10 min. at laboratory temperature and were washed three times with sterile distilled water. Experiments were normally carried out in 50 ml. or 250 ml. conical flasks containing 10 ml. and 50 ml. of culture, respectively. The flasks were incubated in a reciprocal shaking water bath (Gallenkamp Ltd) at a shaking rate of 100 cycles/min. at 30°. Oxygen uptake was measured on a Gilson differential respirometer (Gilson Medical Electronics, Wisconsin, U.S.A.). Respirometer flasks contained 5 mg. dry wt cysts in 2.8 ml. 4% (w/v) mycological peptone (Oxoid Ltd). The gas phase was air, and CO2 was adsorbed by 0.2 ml. 20% (w/v) KOH. Flasks were shaken at 150 cycles/min. at 30°. Acid phosphatase activity was assayed as previously described (Griffiths & Bowen, 1970). Turbidimetric measurements were carried out on a Unicam SP-600 spectrophotometer (Pye Unicam Ltd).

The time taken for excystment varied from one experiment to another but it always occurred between 3 to 5 days following the suspension of the cysts in the appropriate medium. The first sign of activity of the cysts was the appearance of vacuoles in the cytoplasm about 24 h. prior to the subsequent emergence of the amoebae. Most of the cysts germinated within 24 h. of the first appearance of amoebae in the culture, with one amoeba emerging from each cyst. After excystment the cultures contained structurally perfect, but empty, cysts which, like intact cysts, gave a positive reaction for cellulose with Schulze’s solution only after heat fixation. This suggests that neither of the two layers of the cyst wall was ruptured or digested and that the amoebae emerged through a pore in the wall. Such pores have been observed in electron micrographs of cysts by ourselves and by Bowers & Korn (1969).

The oxygen consumption of cysts suspended in growth medium was very low (0.6 to 1.0 µl./mg. dry wt/h.). A slight but significant increase occurred 24 h. prior to the emergence of the amoebae (4.0 µl./mg. dry wt/h.), but it was only after emergence that any large change was observed, when the $Q_o$ increased to a level corresponding to that for vegetative amoebae suspended in growth medium (about 30 µl./mg. dry wt/h.). The extinction and cellular dry weight showed the same pattern as that for oxygen consumption. The dry weight remained constant at 1.5 (±0.1) mg./ml. during the first 72 h. and increased during the 24 h. prior to
emergence to $2.3 \pm 0.1$ mg./ml. Total acid phosphatase activity also increased only during the 24 h. preceding emergence (Table I). This increase was paralleled by a decrease in the percentage free activity of the enzyme. These observations coincided with the appearance of vacuoles in the cytoplasm of the cysts. In view of these results and the finding that a low percentage free phosphatase activity is characteristic of vegetative amoebae, it is tempting to suggest that the changes observed in the pattern of acid phosphatase activity are an indication of renewed endocytotic activity.

Although the changes in the morphological and physiological properties of the cells were only observed in the 24 h. prior to emergence of the amoebae, preliminary experiments have shown that the cysts were committed to excyst after only 12 h. or so in growth medium.

In a system such as this which contains more than two cell types (namely, intact cysts, empty cysts and vegetative amoebae of various morphological types) it was not considered valid to use morphological criteria for measuring excystment. In quantitative experiments, therefore, the time taken for the first appearance of amoebae in a culture would seem to provide the most satisfactory method until further information concerning the physiology of the process is forthcoming.

**Table I. Acid phosphatase activity during excystment**

Acid phosphatase was assayed according to Griffiths & Bowen (1970). Total phosphatase activities were measured on whole cells which had been frozen and thawed once; free phosphatase was measured on fresh unfrozen cells. Activity was expressed as μmoles nitrophenol released/min./ml. culture.

<table>
<thead>
<tr>
<th>Incubation (h.)</th>
<th>Total activity (μmoles nitrophenol/min./ml.)</th>
<th>% Free activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$32 \pm 8.5$</td>
<td>74.5 ± 3.2</td>
</tr>
<tr>
<td>24</td>
<td>$32 \pm 3.0$</td>
<td>89.5 ± 5.3</td>
</tr>
<tr>
<td>48</td>
<td>$44 \pm 8.3$</td>
<td>71.0 ± 9.1</td>
</tr>
<tr>
<td>72</td>
<td>$85 \pm 18.0$</td>
<td>37.0 ± 2.8</td>
</tr>
<tr>
<td>Emergence</td>
<td>$215 \pm 24.4$</td>
<td>15.2 ± 2.8</td>
</tr>
</tbody>
</table>

The excystment of *Hartmannella castellanii* seems to consist of at least three stages: the initiation phase, the pre-emergence phase, and emergence when amoebae appear in the culture. The initiation phase is characterized by the apparent lack of observable events, although the cysts become committed to excyst. Pre-emergent cysts are vacuolated, have a higher dry weight, extinction and total acid phosphatase activity, and a slightly increased respiratory ability. It would seem that the most important changes must occur during the initiation phase, and, as the changes associated with the pre-emergence phase were always observed in the 24 h. or so preceding outgrowth, it is likely that the variability observed from one experiment to another in the time required for emergence is a result of variability in the length of the initiation phase.

It is quite clear that cysts produced by the replacement method differ greatly from those used in other studies (see, for example, Rastogi, Sagar & Agarwala, 1969) in the time taken to excyst. In previous studies of excystment the cysts which constituted the experimental material were produced in 'aged' cultures, and not under conditions of complete carbon and nitrogen starvation as in the experiments reported here. It appears, therefore, that the degree of dormancy exhibited by soil amoeba cysts may be determined by the environmental conditions obtaining during cyst formation, and that excystment could, therefore, represent any of a number of degrees of cryptobiosis.
Short communication

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REFERENCES


