A note on the number of cells in a slug of *Dictyostelium discoideum*

There has been some confusion on how many amoebae are to be found in different size migrating slugs. The first estimates were made by Raper (1941) who separated amoebae from a slug, allowed them to round up (he did not specify in what medium), and measured their diameter. He found that while there was great variation in the size of these spherical amoebae, it can be estimated from his paper that they averaged about 9.7 µm in diameter, and therefore had a mean volume of about 910 μm^3. He then calculated the volumes of different size slugs based on camera lucida drawings, and from this calculated that the number of amoebae per slug range from 189,000 to 770. It is on this basis that one often finds in the literature that migrating slugs have about 100,000 cells.

Using a similar method of estimating cell size, Bonner and Frascella (1953) isolated the amoebae from migrating slugs in standard salt solution (Bonner, 1947) and measured their diameters as spheres. They also found a wide range of sizes and their mean size was approximately 8 µm.

I began to suspect something might be seriously wrong with Raper's cell numbers when I began trapping amoebae in glass capillaries. In very fine capillaries one could see all the amoebae and count how many amoebae were lodged in a given cylindrical volume, and from this calculate the amoeba volume and diameter. It was even possible to measure the number of amoebae that span a given cross section and estimate an average diameter this way. Both methods gave diameters between 3.2 to 3.6 µm, considerably less than that of amoebae set

free in an external medium. However, cells inside a glass capillary might not be comparable to those inside a slug.

This difficulty was overcome by a fortunate circumstance. I remembered that in the study of mini-slugs (Bonner 1998)there was a time-lapse video in which a mini-slug starts as a 3D slug rising into the mineral oil and then comes to the oil-water interface to become 2D. The volume can be estimated for the 3D stage, and the cell number can be accurately measured from the 2D slug where all the cells are visible in a mono-layer. It could be calculated that the volume ~ 78,000 μm^3; cell number ~ 2020; and the volume per cell ~ 38.6 μm^3, giving an average cell diameter of 4.2 μm. This figure is close to that found in the capillary, and again very much smaller than measuring the diameters of the rounded amoebae isolated from a slug.

Using these figures it is possible to recalculate the number of amoebae in Raper's slugs, and there are 14.5 times more cells per slug than he gives. Therefore his slugs range from 2, 740,500 amoebae for his largest to 11,165 for the smallest. In order to make this useful for workers in the field, I have plotted these new cell numbers along with the length of his slugs as a convenient way to estimate the number of amoebae in a slug (Fig. 1).

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