On Cardiac Cells Beating in Culture

The laboratory exercise presented by Debora Weaver (*ABT*, September, 2007) is an excellent recommendation for teaching students sterile technique, cell culture and its usefulness for certain types of experiments.

I wanted to let your readers, especially those in the K-12 arena, know that cell culture of adult or embryonic amphibian tissues is less complicated, making it more available to students and their teachers who do not have the equipment needed for mammalian cell culture. I'm sure improvements in techniques have been made since I did this in the late 1960s, but basically once the mammalian culture medium with an antibiotic was diluted to amphibian tonicity with sterile distilled water, a small piece of lung from an adult or late embryo frog or salamander was placed on a culture coverslip (explant method of obtaining cells – no enzymes needed for cell separation), which was then configured in a hanging drop technique with a larger coverslip covering the relatively large depression in a Romicon or Maximow slide. The edges of the larger coverslip were then sealed against evaporation with petroleum jelly (warm regular glass slide dipped into the jelly and then touched to all edges of the large coverslip). In a day or two the cells grow out onto the smaller culture coverslip that is attached by surface tension to the underside of the larger "chamber" coverslip. Ciliated cells with motile cilia were visible and many mitotic figures were present.

The use of the hanging drop technique means that one does not need an inverted phase contrast microscope to see the cells, i.e. the culture can be viewed using a regular microscope with the light adjusted (condenser down, iris almost closed). Also one does not need a tissue culture incubator because the cultures can be kept at room temperature or in a regular refrigerator. Sterile technique must be used but that does not require a laminar flow hood; we worked in an alcohol swabbed Plexiglas box with one side left open for our hands. All tools were sterilized with flame and the glassware was heat sterilized.

Amphibian cell culture using the explant procedure is a relatively simple way to get a layer of living cells for students to see.

Bob Burns
College of Medicine
University of Arkansas for Medical Sciences
Little Rock, AR 72205

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Understanding Diffusion

The article, "Investigating the Process of Diffusion Using an Analytical Puzzle," that was published in the September, 2007 issue of *The American Biology Teacher* describes a clever approach to teaching some principles of diffusion (Villani et al., 2007). Sadly, this article has serious errors. Specifically, the authors state that the "effect of diameter is inversely proportionate to the rate of diffusion" (p. 412). Exactly the opposite is true – the diffusion rate is ***directly proportional*** to cross-sectional area of the diffusing path. This error is a little surprising considering the authors correctly write the equation for Fick's Law showing "A" (cross-sectional area) in the numerator.

This error is probably the result of the authors' confusion about the term, L, in denominator. The authors erroneously assumed that "*l* is the distance the dye travels, which we are observing in our demonstration today" (Fig. 3). This term actually refers to the distance that separates two points of different concentration (i.e., the concentration gradient) such as the thickness of a membrane. In reality, the demonstration is measuring the rate of transport or flux density (F), which also can be symbolized by the letter "J" and is commonly expressed in units of mol m^{-2} s^{-1}.

One relatively simple way that the authors could have demonstrated that the diffusion rate or flux density is directly proportional to cross-sectional area is to calculate, using the equation for the volume of a cylinder ($=\pi r^2 l$), the volume of agar permeated by the dye. If we measure the size of the images in Fig. 4A, the smaller tube is approximately 4.5 mm wide and the dye migrated 38 mm, which means that the total volume of agar into which the dye diffused is 604.4 mm^3. Performing the same calculations for the larger tube (7 x 32 mm) the volume of agar permeated by dye is 1231.5 mm^3. These results clearly support the predictions of Fick's Law. The authors' excellent analogy of herding a cat down a hallway explains why the dye diffuses further in the smaller tube (Fig. 4A). Unfortunately, this analogy isn't applicable to Fick's Law because it doesn't consider ***how much*** dye diffuses.

If a teacher corrects the mistakes in this article, this exercise will provide a nice visual demonstration of diffusion. However, this article provides further evidence that students and teachers may have difficulties understanding diffusion as past articles in this journal have shown (Vogel, 1994).

Stephen G. Saupe
College of St. Benedict
Biology Department
St. Joseph, MN 56374
ssaupe@csbsju.edu

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