Hydra: Research Methods

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Chapter 20

Dissociating Hydra Tissue into Single Cells by the Maceration Technique

Charles N. David

PURPOSE

To rapidly and completely dissociate hydra tissue into its individual cells and to fix them for identification and counting.

INTRODUCTION

Quantitative data on the abundance and distribution of each cell type in hydra is essential to investigating a variety of problems concerning the cell biology and morphology of hydra. Obtaining such data in histological sections is difficult and time-consuming. We have, therefore, developed a maceration technique for rapidly and completely dissociating hydra tissue into individual cells (David, 1973). Each cell type is readily distinguishable under phase microscopy in such macerations, and its abundance can be determined by counting.

MATERIALS

The maceration solution is glycerin/glacial acetic acid/water (1:1:13). As a fixative, use formaldehyde (8%) or osmium tetroxide (1%). "Subbed"

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microscope slides are prepared by dipping washed slides in a solution of 5% (w/v) gelatin and 0.5% (w/v) chrome alum. Dry the slides before use. Other materials are 1% Tween 80 detergent (Sigma), small disposable polyethylene tubes (4 ml), Falcon Microtest dish (96 0.4-ml wells), and hemoctyometer.

PROCEDURES

Dissociating Tissue

Place pieces of hydra or whole hydra in disposable polyethylene tubes, draw off excess medium with a Pasteur pipet and add about 0.1 ml maceration solution per hydra. Let the tissue soak for 2–3 min and then dissociate it by gently shaking the tube (tapping it on fingernail or table works well). It is best to carry out the procedure under a dissecting microscope in order to monitor the extent of the dissociation.

Tissue from the gastric region gives a homogeneous turbid cell suspension without noticeable clumps with very little shaking. Samples which include hypostome, basal disk, or tentacles require longer soaking and more vigorous shaking to dissociate; even vigorous shaking may not yield a suspension free of clumps. However, the cells in small clumps can usually be identified and counted when the preparations are examined in a microscope.

There are differences between species in the susceptibility to maceration. *Hydra attenuata* macerates easily; *Hydra viridis*, by comparison, requires more vigorous dissociation to yield good cell suspensions.

Fixing Cells and Preparing Cell Spreads for Counting

When dissociation is complete, add fixative. To examine cells and determine cell counts, transfer an aliquot of the suspension to a hemocytometer or to a subbed microscope slide on which a small drop of detergent has been placed. Spread the drop over an area of 1–3 cm^2 on the slide, depending on the concentration of cells in the suspension; the more cells, the greater the area. For example, if five hydra (about 250,000 total cells) are macerated in a final volume of 1.0 ml maceration solution plus fixative, spread 0.1 ml of the suspension containing 25,000 cells over an area of 200 mm^2 on the slide to yield a density of 125 cells/mm^2. This density corresponds to about 40 cells per field when viewed with a 25-power objective lens. For most purposes this density of cells on a slide is convenient for counting. With lower densities, one has to scan large areas to find enough cells when minor cell types such as nerves are being scored. (For illustrations of individual cells, see Chapter 1).

Microtechnique for Individual Pieces of Tissue

To dissociate individual small pieces of tissue, use the wells in Microtest dishes (volume 0.4 ml) for maceration in place of tubes. While observing the whole process under a dissecting microscope, transfer a piece of tissue to the well, draw off excess medium with a pipet, and add one or two drops of maceration solution. To shake the microtiter dish, slide it back and forth on the bench top, banging it against an object like a bottle cap. This sort of vigorous motion is required to stir the small volume in the well. After dissociation is complete, add one drop of fixative and transfer the entire contents of the well with a Pasteur pipet to a slide and spread it over about 1 cm^2. To rinse the last few cells out of the well, add another drop of fixative, shake the well, and transfer the rinse to the slide.

After spreading, place slides on a level surface so that the solution dries evenly; otherwise cells which settle out of the suspension slowly will accumulate on the last part of the suspension to dry on a tilted slide. Such assymmetric drying can seriously distort cell counts.

Counting Cells

After drying on slides, macerations can be examined in several ways. To count cell types, place a drop of 10% glycerin and a coverslip on the slide and examine the preparation using a 25- or 40-power objective lens in a phase contrast microscope. All cell types can be easily recognized with phase optics, and no cells are lost from the slide. To preserve the preparation for a period of months, ring it with a coat of lacquer (nail polish).

To count cells on slides, determine the boundaries of the cell spread using a stage micrometer. Then count all the cells in several complete passes across the spread. From the width of the field and the dimensions of the spread, calculate the total cells on the slide. If a known volume of cell suspension was dried on the slide, calculate the cell concentration in the original suspension. This technique is particularly useful for counting minor cell types such as nerves since large areas of the spread of cells can be easily scanned.

Autoradiography

Macerations on subbed slides are suitable for autoradiography using stripping film or dipping film. Before covering the slides with film, rinse them for 5–10 min in water in order to remove the glycerin and formaldehyde, which cause chemical "exposure" of the film and make scoring labeled cells difficult to carry out. To score labeled cells in autoradiographs, develop the film and dry the slides. Then place a drop of water and a coverslip over the film and examine the preparation by phase contrast microscopy.

Staining

Macerations are suitable for staining by a variety of standard histological techniques. For example, Feulgen-stained preparations can be used for counting mitotic cells and determining the DNA content of individual nuclei by microspectrophotometry (David and Campbell, 1972). Although cells in macerations stick to subbed slides fairly well, the frequent dipping and washing procedures involved in staining may cause some loss of cells from the slides.

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