Hydra: Research Methods

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Chapter 25

Incorporating [^{3}H]Thymidine into Hydra by Microinjection

Charles N. David

PURPOSE

To label hydra by injecting [^{3}H]thymidine into the gastric cavity (Campbell, 1965; David and Campbell, 1972).

INTRODUCTION

Hydra, because of their low permeability, cannot be effectively labeled with radioactive materials by the usual method of soaking animals in a solution containing the isotope. Injection of [^{3}H]thymidine into the gastric cavity offers several advantages over soaking hydra in medium containing isotope. (1) [^{3}H]Thymidine is taken up 10 to 20 times more effectively from the gastric cavity than from the external medium, and only small amounts of isotope are required for injection. (2) The injected [^{3}H]thymidine labels primarily hydra cells and not bacteria, which exist in large numbers on the outside of hydra. By comparison, >95% of the label is incorporated into bacterial DNA when hydra are soaked in [^{3}H]thymidine. (3) Injection of [^{3}H]thymidine yields a natural pulse label. A single injection is taken up within 45 min in unfed animals and within 90 min in fed animals.

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MATERIALS

Hamilton syringe (10 or 25 μl; Hamilton Co., Reno, NV), polyethylene tubing (Intramedic PE 50; Clay-Adams, Parsippany, NJ).

PROCEDURES

Preparing Polyethylene Needles

Polyethylene needles are superior to glass needles for injecting hydra because they are slightly flexible. Prepare the needles by warming polyethylene tubing (PE 50) near a small flame such as the pilot light of a Bunsen burner. When the tubing starts to melt, remove it from the flame and draw it out. The short melted section pulls out into a thin tube which hardens almost immediately. Cut the tubing with a razor blade on an angle at the point where its diameter is about 0.1 mm. Attach the needle to the end of a Hamilton syringe.

Filling the Needle

Remove an appropriate small amount (20–100 μl) of isotope from the stock solution and place it on the bottom of a 20-mm plastic Petri dish. The solution forms a round drop since the plastic surface is hydrophobic. Keep the dish covered to avoid evaporation. Draw up the isotope solution into the polyethylene needle but not into the syringe itself. Discard the needle at the end of the experiment.

Injection Procedure

Place 5–10 hydra in 10 ml medium in a 50-mm plastic Petri dish. Move the hydra to the center of the dish, loosen them from the bottom, and wait for them to stretch out a bit. During injection, view the hydra under low power in a dissecting microscope. With tweezers in one hand, position a hydra and hold it lightly behind the tentacles. With the other hand bring the Hamilton syringe into position and insert the needle into the mouth of the animal. The insertion is generally easy to do with thin needles and does not normally cause the animal to contract much or to open its mouth. Once the needle is inserted, gently press the plunger of the syringe to expel a small volume of solution into the hydra's gastric cavity. Estimate the amount injected by watching the movement of the meniscus of the solution in the needle; usually 0.25 μl occupies a length of 1 mm.

After the hydra swells up, wait a few second before withdrawing the needle. The mouth usually closes tightly as the needle is withdrawn and the hydra remain somewhat swollen. If individual animals contract immediately upon withdrawal of the needle, reinject them a minute or two later. With practice one can inject two to three hydra per minute.

For individuals with unsteady hand, the Hamilton syringe may be mounted in a motor-driven syringe pump activated with a foot pedal. If the injection volume does not need to be controlled, the injection needle may be connected directly to a mouth pipet and the isotope expelled by blowing gently.

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