

PHAGOCYTOSIS BY CLAM HEMOCYTES

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All multicellular animals have a cellular defense system that aids the animal in defending itself against foreign material. In some invertebrates (i.e. the hard clam), these wandering cells are also involved in feeding (intracellular digestion). The wandering cells, called hemocytes, can migrate throughout the body; they may be found in large numbers in open sinuses such as the clam adductor muscles.

The hemocytes have the ability to internalize foreign material by a process called phagocytosis. They 'sense' the foreign material, migrate towards it (chemotaxis), attach to the material (adherence) and internalize it by extending pseudopods and pulling the material into the hemocyte where it will reside surrounded by host cell membrane forming a phagocytic vacuole.

MATERIALS

1. Living clam from grocery- e.g. *Mercenaria mercenaria* (quahog, hard-shelled clam)
2. Neutral red or brom thymol blue solution in canned clam juice or Ringer's solution (1:10,000)
3. Clam Ringer's solution (in distilled water)
 - Potassium chloride, 0.014% (all values w/v)
 - Sodium chloride, 0.65%
 - Calcium chloride, 0.012%
 - Sodium bicarbonate, 0.02%
4. Baker's yeast, dry powder or fresh cake
5. Wood file (coarse)
6. 1ml hypodermic syringe with 18g (1 inch) needle
7. Glass slide and coverslip
8. Compound microscope with e.g. 10X ocular and 40X objective lenses (brightfield illumination)
9. Small pipet, e.g. Pasteur pipet or polypropylene transfer pipet
10. Centrifuge and tubes
11. (optional) Microcentrifuge and 1.5ml centrifuge tubes

METHODS

1. Boil a small amount of yeast in dilute neutral red or brom thymol blue solution for 5 min. Cool. Rinse cells by centrifugation and resuspension in clam juice or clam Ringer's solution.
2. With a file grind down the bivalves where they meet near the position of the anterior or posterior adductor muscle until an opening between the valves large enough to insert a hypodermic needle is produced.
3. Insert the syringe needle into the adductor muscle. Slowly withdraw up to 1 ml of hemolymph.
4. (optional) Concentrate the hemocytes. Transfer the hemolymph to the microcentrifuge tube, spin 1 min at half speed. Carefully decant the supernatant liquid and gently suspend the pellet in 0.1 ml clam juice or clam Ringer's solution. If hemocytes are not active, try spinning at a slower speed or omit.
5. Place a drop of suspended hemocytes on a clean glass microscope slide. Add a very small amount of stained yeast suspension. Gently place a coverslip over the preparation.

6. Observe hemocytes with brightfield illumination, increasing contrast by cutting down light with the substage iris diaphragm. Look for almost transparent, unpigmented cells having an irregular shape with small, pointy projections. As the hemocytes settle, they will adhere to the slide and will then be capable of migrating toward and engulfing individual yeast cells. Most hemocytes will be able to engulf yeast, some as many as 20 yeast cells. One needs patience. Hemocytes move very slowly. Allow at least one-half hour to observe phagocytosis.

REFERENCES

Cited above.

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