Cell Biology Laboratory Exercise - Active Transport in Cockroach Malpighian Tubules

Active transport is a process which is vital to cells, and by extension, to whole organisms in order to maintain differences between themselves and the environment with regard to concentrations of many substances. One of the areas where active transport is extremely important is in the maintenance of appropriate osmotic conditions and the elimination of waste materials. Individual cells perform these regulatory activities; collections of these cells form organs that specialize in them.

Many systems can be used to demonstrate the characteristics of active transport processes, such as its specificity for particular substrates, the phenomenon of competition between similar molecules, its dependence on metabolic energy and susceptibility to "metabolic poisons" such as dinitrophenol, and its sensitivity to temperature changes. Two classic preparations for laboratory exercises include the kidney tubules of goldfish and the Malpighian tubules of insects. We will use the latter for this exercise. Transport can be visualized if colored vital dyes are used as the material to be moved; as dye accumulates in the lumen of the tubule, the color change can be followed. Careful observation can reveal relative differences in transport rates under different conditions. Dyes that are commonly used for such demonstration include phenol red, neutral red, and bromcrescol green. They have the added advantage that they change color depending on the pH of the medium, giving us information about the luminal pH of the tubules.

Among insects, diplopods, chilopods and many arachnids, one finds Malpighian tubules, elongated outpocketings of the hindgut, as specialized excretory organs. The tubules vary greatly in number, from a single pair to over 100 in different species, and they are correspondingly varied in form. Nitrogenous wastes, often with a high percentage of uric acid, are passed into the tubules from the hemolymph in solution. Excess water is reabsorbed, either in the tubules or in the rectum, leaving the urine and fecal matter in a more-or-less dry form.

Our experimental preparation will be the Malpighian tubules of the cockroach, *Periplaneta americana*, in which there are 60 or more tubules. These tubules are yellow in color and possess considerable motility within the body cavity. They are also very delicate and considerable care is required in dissecting them from the animal and placing them in test solutions. If they are crushed or stretched, they will be damaged and transport processes will cease. **The use of a dissecting microscope with good lighting is critical to visualizing clearly what you need to see - no matter how near-sighted you might be.** You also need to utilize clean, fine dissecting instruments to be able to separate the tubules from other structures. There will be several tubular organs visible within the abdomen of the animal. The fat bodies will be elongated and milky-white, the tracheoles will be translucent and silvery, and the gut will be large and brown. Neither the tracheoles not fat bodies will possess any inherent motility; the gut will likely be moving rather enthusiastically. The remaining tubular structures, the Malpighian tubules, will be translucent yellow and often moving about in a wavelike manner.

The exercise has several objectives:

- to observe the appearance and movement of Malpighian tubules within the hemocoel of the cockroach,
- to observe the process of dye transport into the Malpighian tubules,
- to observe the competitive nature of the transport process,
- to observe the effects of metabolic inhibitors on the transport process, and
- to observe the effects of temperature variation on transport rates.

Procedure:

1. Decapitate an anesthetized adult cockroach (*Periplaneta americana*) and pin the body securely in a wax dissecting dish.

2. Open the abdomen by cutting carefully around the edges of the ventral abdominal wall and removing it. As you lift it off, be careful to tease apart the soft tissues and the exoskeleton to which they tend to adhere. Flood the body cavity with insect saline and **keep it moist** throughout the exercise.

3. **Carefully,** remove as many of the tubules as possible by cutting them away from the hindgut and transfer them to a dish of fresh insect saline. **Do not stretch or crush the tubules if you expect them to work for you!** Transfer small quantities from this dish to individual test solutions in the wells of a spot plate or on depression slides. Keep tubules moist at all times using insect saline.

4. Add a drop or two of neutral red solution to one group of the tubules and observe with a dissecting microscope the accumulation of dye in the tubule lumen. Notice the time required for you to perceive the dye initially and, also, how long the transport can be followed. Initial transport can happen very quickly so **be prepared**. Note the color of the lumen; what can you infer about its pH noting the colors of the dyes in the acidic and basic solutions provided for demonstration?

5. To demonstrate the selectivity of the uptake mechanism, add a mixture of neutral red and bromcrescol green. What is the color of the tubule lumen? What is the time course for uptake of the dye(s)?

6. To demonstrate the effect of metabolic inhibitors on the transport process, add a drop of DNP (dinitrophenol) solution and a drop of neutral red. What is the effect? Repeat this with a mixture of IAA (iodoacetic acid) and neutral red on a fresh tubule preparation. What is the effect? Note any differences in the time courses.

7. Compare the rates of dye accumulation at 0° C with that at room temperature (about 25° C.) Calculate a Q_{10} for the transport process. (Q_{10} is the ratio of the rate of a process at one temperature with its rate at a temperature 10° C lower.)

Points to Ponder (as you write your lab report):

Why would Malpighian tubules be expected to have such delicate structure and to be moving within the hemocoel?

What would you propose as the explanation for the pH observed in the tubule lumen?

Why is only one dye taken up when two are present?

What are the specific mechanisms of action of DNP and IAA? Would you suspect, based on these respective mechanisms, that one would be a more effective inhibitor than the other? Why?

How would you explain the results of comparing uptake rates at different temperatures?

What "actor(s)" is/are responsible for all this uptake work, anyway?