Chemiluminescent Detection of Diffusion

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Grade Level:
High School

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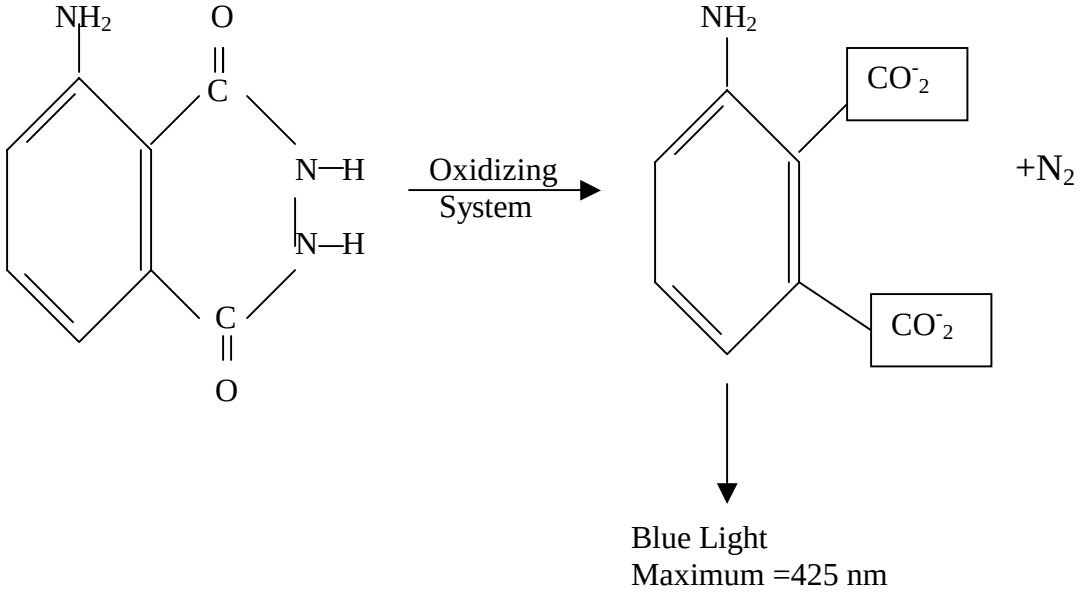
Introduction:

Chemiluminescence is observed when light is emitted from a chemical reaction. Generally, the intensity of the light is observed to increase initially and later decrease with time as the reactants are consumed. Two of the most efficient and best-known chemiluminescent reactions is the firefly system and the glow of solid phosphorus in air. In research,Western and Southern blots are developed with chemiluminescence. Chemiluminescence is used to detect low abundance protein in complex cell and tissue samples. Other detection systems used in research include ultraviolet emission for RNA analysis, and for greater sensitivity radioactive isotopes ($^{125}1$, ^{3}H, ^{32}p) are used to label antibodies. The use of radioactive isotopes to label antibodies is a beneficial use of radiation; however it requires special handling, training, and disposal. Chemiluninescence provides high sensitivity for detecting enzyme catalyst, antibodies, and metals without the disposal concerns associated with the use of radioactive isotopes.

The oxidation of luminol, (5-amino-2, 3-dihydrophthalazine-l, 4-dione) one of the more commonly known chemiluminescent reaction, will be used in this lab to measure diffusion through dialysis tubing. According to the law of **diffusion**, molecules tend to move from areas of higher concentration to areas of lower concentration. A membrane through which substances diffuse at different rates is called **selectively permeable membrane**. Small solute molecules and water molecules can move freely through a selectively permeable membrane, but larger molecules will pass more slowly, or not at all.

In this experiment solutes are separated by a selectively permeable membrane; starch and luminol are placed inside a dialysis bag. The dialysis bag is immersed in a beaker of water. Molecules can move both ways across the dialysis bag membrane if they are small enough. Samples of the water surrounding the dialysis bag will be tested through chemiluminescence and with I_2KI (Lugol's solution.) The chemiluminescence reaction involves the oxidation of luminol to form an excited amino phthalate ion in an excited electronic state, which fluoresces.



Students will then quantitate the luminescence by recording the light emitted on photographic paper.

Objectives:
Students will be able to:

- observe the results of diffusion through a selectively permeable membrane.
- monitor the concentration of the solute in the water surrounding the dialysis bag through chemiluminescence.
- learn the simple test for starch.
- quantitate the chemiluminescence by exposure to photographic paper .

Materials:

- cellophane dialysis tubing
- glass rod
- beakers, 250 ml , 150 ml
- string
- starch solution
- luminol solution
- bleach solution
- graduated cylinder, 25 ml
- test tubes
- thin stem pipets
- Lugol's iodine solution
- test tube Rack
- cardboard box 8" x 8" x 6" (top removed)
- Multigrade III Photographic Paper

Procedure:

Part I: Diffusion and Chemilunimescence Quantitated by Time

1. Obtain three test tubes and label starch, luminol, and tap water. Using pipettes, add equal volumes of starch and Lugo's iodine solution to one test tube and stir. Observe and record observations in data table. After rinsing the pipettes thoroughly, repeat the procedure twice testing Lugol's iodine solution with luminol and tap water.
2. Rinse the three test tubes and pipettes thoroughly. Using pipettes, add equal volume of tap water, luminol, and starch to the labeled test tubes. In a darkened classroom, add equal volume of the bleach solution to each test tube, observe and record observations.
3. Add 200 ml of cold tap water to a 250 ml beaker. Obtain four clean test tubes and pipette; label test tubes 1, 2, 3, and 4 and place the test tubes in a test tube rack.
4. Soak a 15 cm length of cellophane dialysis tubing in a 150 ml beaker of water for a few minutes. Rub the ends of the dialysis tubing between your thumb and index finger until the

ends separate. Insert a glass rod into the tubing to hold it open. Twist one end and tie it tightly with string.

5. Remove the glass rod and fill the dialysis bag half full with starch solution. Then add 10 ml of luminol solution. Tie the top of the bag with string, leaving a loose piece of string 10 cm long. Rinse the outside of the bag with water.
6. Immerse the filled dialysis bag in the 250 ml beaker of water. Leave the string outside the beaker so that you may remove the bag from the water. After 10 minutes, using a pipette, take a 2 ml sample from the bottom of the beaker. Add the sample to test tube 1 in your test tube rack. Repeat this procedure three times at 10-minute intervals for the next 30 minutes.
7. Fill four pipettes with 1 ml of bleach solution. Obtain a stopclock or watch with a second hand. In a darkened classroom add one pipette of bleach to sample 1 from step 6. Observe the intensity of the luminescence (if present) and measure the length of time the luminescence lasts. Record observations. Repeat this procedure with the remaining three test tubes and bleach solution.
8. Leave the dialysis bag immersed in the water overnight.
9. Next day, add 200 ml of water to a 250 ml beaker. Mix 15 mi of Lugol's iodine solution in the water. Remove the dialysis bag from the first beaker and rinse the bag with running water. Immerse the dialysis bag in the beaker containing the Lugol's iodine solution, and set it aside for 20 minutes.
10. Observe the colors of the solution in the cellophane dialysis bag and in the beaker.

Part II Diffusion and Chemiluminescence Quantitated with Photographic Paper

1. Obtain three test tubes and label starch, luminol, and tap water. Using pipettes, add equal volumes of starch and Lugol's iodine solution to one test tube and stir. Observe and record observations in data table. After rinsing the pipettes thoroughly, repeat the procedure twice testing Lugol's iodine solution with luminol and tap water.
2. Rinse the three test tubes and pipettes thoroughly. Using pipettes, add equal volumes of tap water, luminol, and starch to the labeled test tubes. In a darkened classroom, add equal volume of the bleach solution to each test tube, observe and record observations.
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6. Immerse the filled dialysis bag in the beaker of water. Leave the string outside the beaker so that you may remove the bag from the water. After 10 minutes using a. pipettes, take a two ml sample from the bottom of the beaker. Add the sample to test tube one in your test tube rack. Repeat this procedure three times at 10-minute intervals for the next 30 minutes.
7. Fill four pipettes with 1 ml of bleach solution and place them in a beaker with the stems pointing upward. Place the four test tubes containing samples from step 6 in a test tube rack that allows the bottom half of the test tube to be exposed to light (for example a wire test

tube rack.) Place the test tube rack at one end of the cardboard box so that the test tube is no more than 3 cm from the end of the box. Place the beaker of pipettes containing the bleach in the box also (See Figure 1).

8. Obtain a piece of black heavy construction paper or poster with dimensions equal to the side of the box that the test tube rack is touching. The black paper should be 10 cm higher than the box height. In the middle of the shortest side of the paper, 1 cm up from the edge, draw a rectangular window that requires an area no greater than 2.5 cm x 1 cm. Using an xacto knife, cut out the window. (See Figure 2). Place a 10 cm piece of masking tape loosely about 12 cm above the window. (See Figure 3). Place the black paper between the test tube rack and the side of the box. The window should be positioned so that it is touching the base of the test tube 1 in the center of the rack (refer to Figure 1).
9. Transfer the entire box to the dark room, which is illuminated with safety light. The photographic paper has already been pre- cut in the dark room and is stored in its original packaging and box on the counter.
10. After your eyes have adapted to the dark room, remove one piece of photographic paper. Using a pencil, record your initials and number 1 on the non-glossy side of the paper. Remove the black paper from the box. Use the masking tape to mount the photographic paper on the back-side of the black construction paper so that the glossy side will face the test tube through the window. The photographic paper should cover the window. Place the black paper with the mounted photographic paper back into the cardboard box so that it is touching the base of test tube 1 located in the center of the rack. Add one pipette of bleach to test tube 1. Allow the test tube and photographic paper to remain in position until the luminescence has totally ceased. At this time lift the black paper from the box and remove the photographic paper. Place the photographic paper in an empty photographic paper box within the original packaging. Remove test tube 1 from center position.
11. Place test tube 2 in center position and repeat step 10.
12. Repeat step 10 for samples 3 and 4.
13. The photographic paper is now ready to be developed by your school's photography class, photography students enrolled in your science class, or your science class.
14. Leave the dialysis bag immersed in the water overnight.
15. Next day, add 200 ml of water to a 250 ml beaker. Mix 15 ml of Lugol's iodine solution in the water. Remove the dialysis bag from the first beaker and rinse the bag with running water. Immerse the dialysis bag in the beaker containing the Lugol's iodine solution, and set it aside for 20 minutes.
16. Observe the colors of the solution in the cellophane dialysis bag and in the beaker.

Analysis and Interpretations

1. Where are the controls in this experiment?
2. Lugo's reagent is used to test for the presence of which substance? What color change occurs in the presence of this substance?
3. Bleach solution is used to test for the presence of which type of substance? What was observed in the presence of this substance?
4. List some applications of chemiluminescence in everyday life. (Consult additional sources of information.)
5. Which substance or substances left the bag? Which substances entered the dialysis bag? Give evidence for your answer.

6. Which substances were not able to pass through the dialysis bag? What changes to the selectively permeable membrane will alleviate this problem?
7. Use the terms hypertonic, hypotonic, or isotonic to describe the solutions inside the dialysis bag and in the beaker at the beginning of the experiment. (Consult additional sources of information.)
8. Do your results on photographic paper indicate diffusion? Do your results on photographic paper suggest a steady change in concentration as a function of time? Explain.

For Further Investigation:

1. To further quantitate this lab, students may use an exposure meter and assign numbers to the exposure of the photographic paper.
2. To enhance creativity, students may change the shape of the window, or place a negative between the chemiluminescence and the photographic paper. This creates interesting images on the photographic paper.
3. With the help of my mentor, have students visit the summer research facility and scan their photographic papers for densitometry.

References:

1. Wannlund, I.; DeLuca, M. In Bioluminescence and ChemiJuminescence, Basic Chemistry and Analytical Applications; Academic: New York. 1981. 00.693 -696.

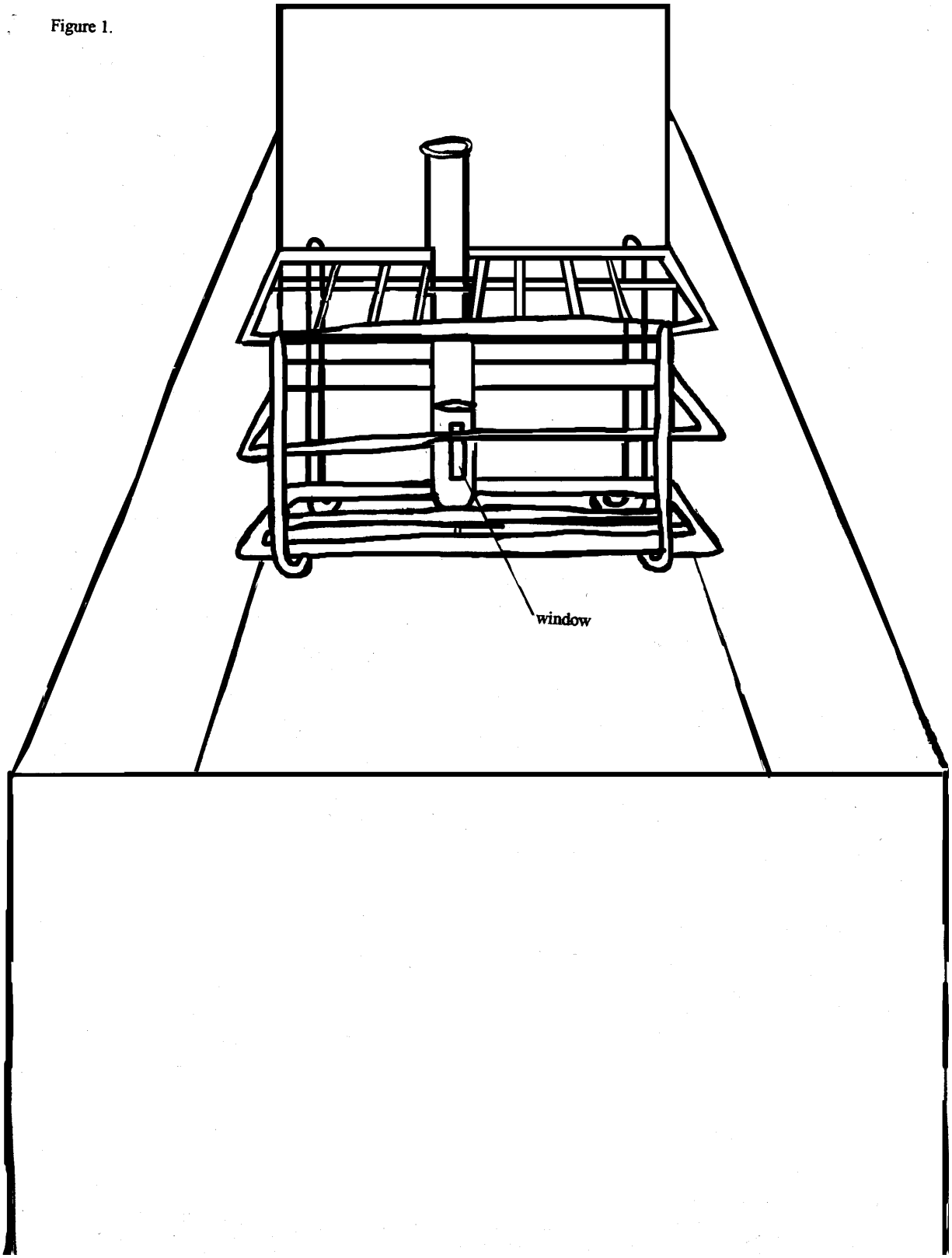
Pre-Lab Preparation for Teacher:
This experiment is appropriate for the study of diffusion, light emission, and the understanding of lightsticks used by scuba divers and the celebration of Hal1oween.

1. Luminol may be purchased from Flinn Scientific, Inc., Batavia, Illinois.
    a. **Luminol solution:** Prepare 1 M sodium hydroxide by dissolving 4 g of sodium hydroxide in 100 ml water. Dissolve 0.1g luminol in 100 ml of 1 M sodium hydroxide. Add 10 ml 3% hydrogen peroxide to this solution. Stir, cover container tightly, and store in the refrigerator.
    b. **Bleach solution:** Dilute 20 ml bleach to 100 ml water and stir. Bleach solution may be replaced with horseradish. Peel and grate the horseradish. Extract the enzyme by suspending the horseradish in small amount of cold water and stirring. Keep the horseradish suspension in the refrigerator. The enzyme's ability to react with luminol and produce chemiluminescence decreases with time.
        i. Potassium iron (ill) cyanide solution may also be substituted for the bleach solution. It produces less intensity.

Part I: the experiment may be completed entirely in the lab.

Part II: lab requires more laboratory skills of students and requires the use of a dark room.
This lab can be an introduction to photographic developing. The dimensions of the cardboard box may differ from those listed in materials. However, the height of a shoebox is too low. The photographic paper may be pre-cut 11 cm x 12 cm in the dark room.

Figure 1.



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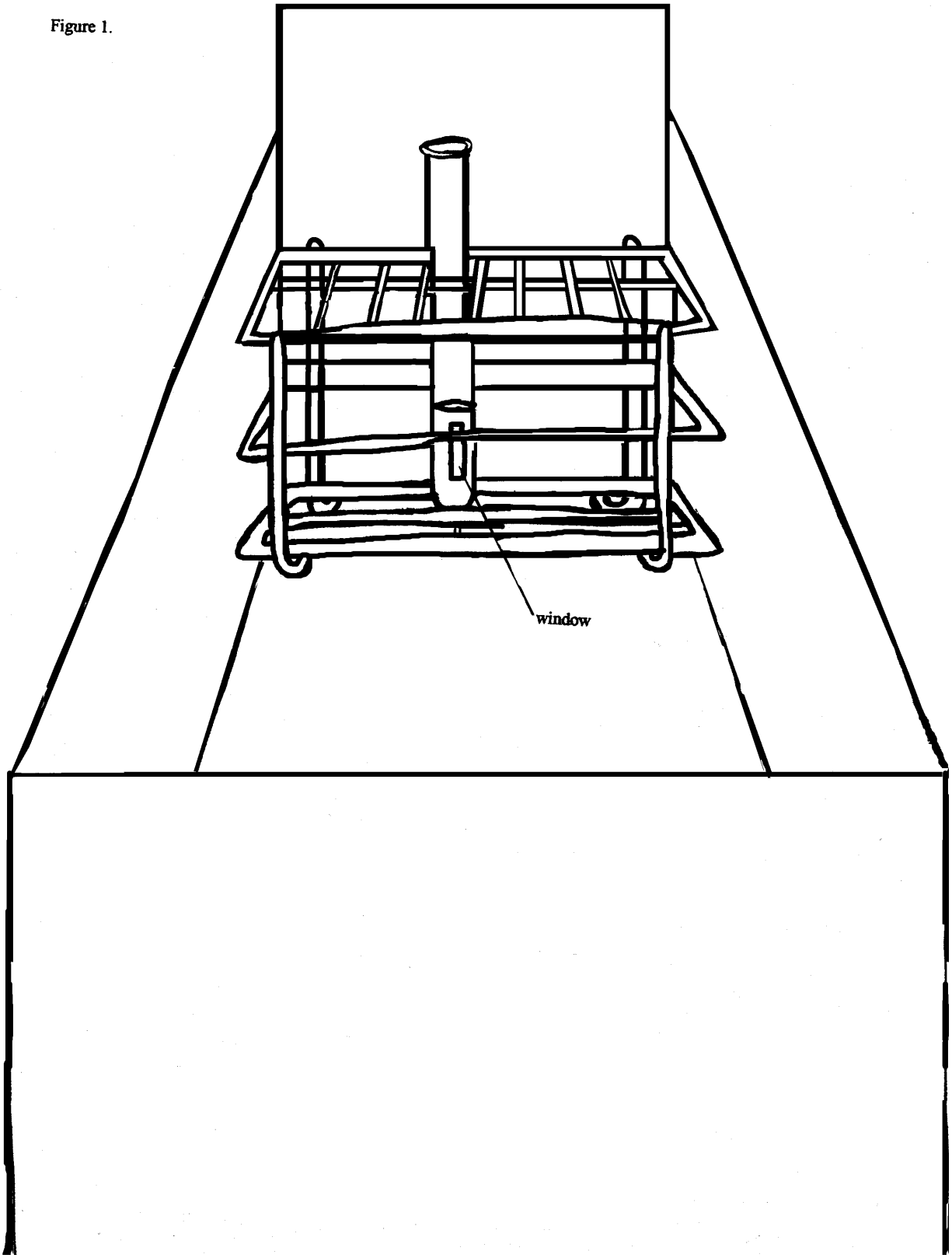

Figure 2. (side of black paper facing test tube rack)

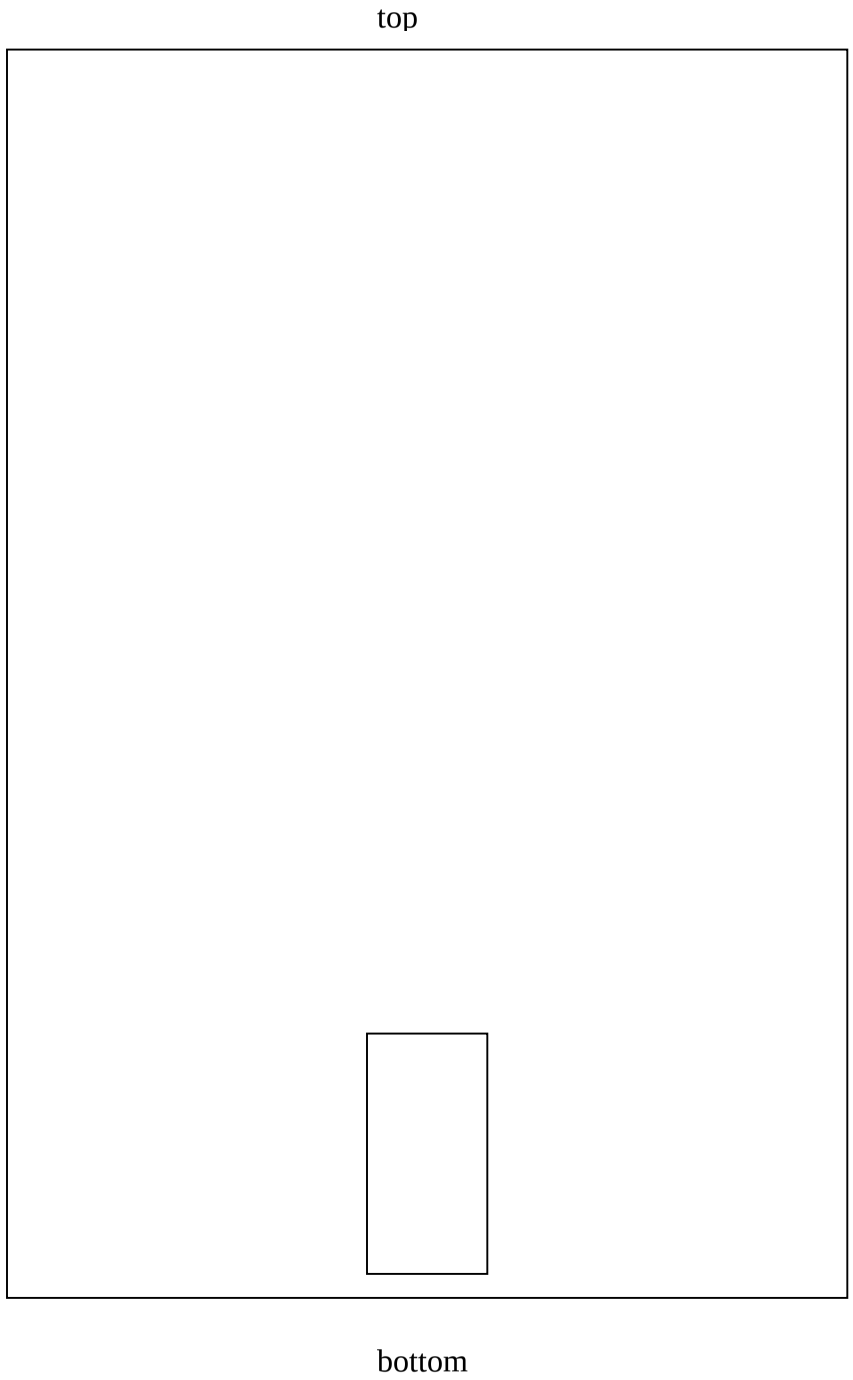

Figure 3. (side of black paper against cardboard box)