# Death by Osmosis: An interactive, investigative laboratory exercise


## *William M. Beachly*

Department of Biology
Hastings College
700 Turner Avenue
Hastings, NE 68901
wbeachly@hastings.edu


William Beachly is an assistant professor who teaches courses in Principles of Biology (a two-semester major's course), Human Physiology, Ecology, Animal Behavior, and Invertebrates.

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ABSTRACT: I devised this exercise to enhance interest and student involvement in what had become a rather routine laboratory demonstration of diffusion and osmosis. We begin the lab by having two student volunteers reading the short play "Death by Osmosis" in the roles of Sherlock Holmes and Dr. Watson. Student groups have materials sufficient to re-enact the murder weapon and a worksheet that challenges them to understand the processes involved. Different groups compare their hypotheses as to how and why the weapon works. After this discussion, to focus their efforts I read Holmes' soliloquy as I play a videotape showing the rubber-gloved hands of the murderer going through the necessary steps. This demonstrates the techniques they must follow in their own investigation. Such a videotape is easily made using a Flexcam, Vizcam, or similar desktop video camera. I enhanced the drama by overdubbing a haunting musical score (Phillip Glass' "Facades" from Glassworks). Now it's up to the students to solve the murder by reenactment of the weapon. The key question being, "how long will it take to tip over?" I have included the play and soliloquy, helpful figures, student worksheets, and notes to the instructor.

## Death by Osmosis

### Scene 1: The cramped and stuffy study of Professor Plum.

**Narrator:** Professor Plum was found dead in his favorite chair at 6 AM by his housekeeper who summoned the constable. Scotland Yard has called in the great detective Sherlock Holmes and his assistant, Dr. Watson to investigate.

**Holmes:** (in a cool, matter-of-fact tone) Professor Plum has died of osmosis.

**Watson:** Great blazes, Holmes! What does that mean?

**Holmes:** I mean that osmosis was both the cause of death and the murder weapon.

**Watson:** Aren't those one in the same?

**Holmes:** Not necessarily. Nearly every cell in his body is swollen with fluid. The poor chap's blood pressure dropped and his heart arrested, but not before he was able to scribble three letters in his notebook: H C N.

**Watson:** What does that mean? What does it have to do with osmosis?

[Pause now for student input...]

**Holmes:** It's cyanide, hydrogen cyanide to be precise. No doubt his last sensation was a smell of almonds. It has quite a bit to do with osmosis.

**Watson:** You mean he was poisoned? There's no sign of food or drink around.

**Holmes:** My dear *Doctor* Watson, you should really know that there is an imbalance of ions across the cell membrane. Protein molecules with mostly negative charges are trapped inside. Potassium is the abundant cation inside, Sodium is the abundant cation outside, and anions like Chloride are repelled by the negative proteins.

**Watson:** Ah, yes! An imbalance maintained by the Sodium-Potassium antiport which pumps three Sodium ions out for every two Potassium ions it pumps in.

**Holmes:** And what molecule does that pump require to work?

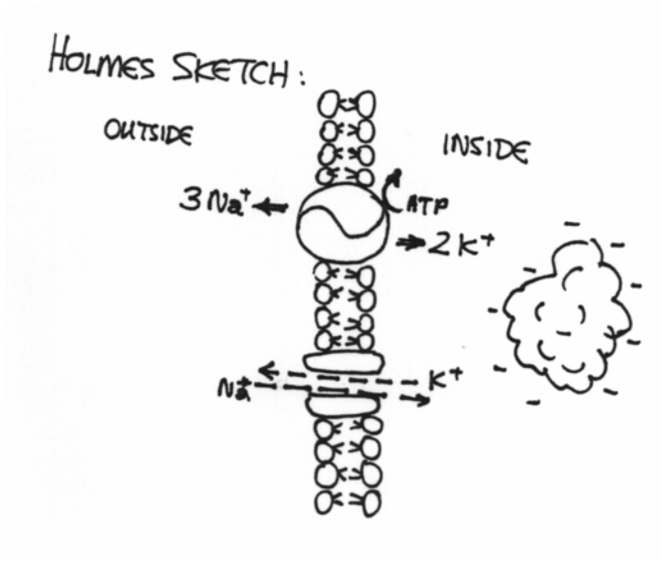
[Pause now for student input...]

**Watson:** By Jove! I've got it now. Since cyanide blocks the final step of electron transport in the mitochondria, the whole process of respiration ceases! No oxygen is used, no more ATP can be made. It's an energy crisis!

**Holmes:** Yes, it's like suffocation although oxygen is abundant, none of it can be reduced. You will not find the usual signs of respiratory arrest.

**Watson:** I see. But I still don't quite get the picture about the osmosis part.

**Holmes:** Allow me to illustrate: [display overhead of Holmes' sketch]




**Watson:** So when the pump, which requires ATP, stops, Sodium diffuses in along it's concentration gradient and also a favorable electrochemical gradient. This creates a much greater osmotic potential inside the cell where the environment is hypertonic to the outside...

**Holmes:** And water follows by osmosis. Eventually the cell lyses and dies. Also, without ATP, rigor mortis would set in more quickly than in a typical murder. Perhaps in a matter of an hour. And since proteolytic enzymes would be liberated from the ruptured cells, the cross-arms between actin and myosin would also break down faster, perhaps in three to four hours.

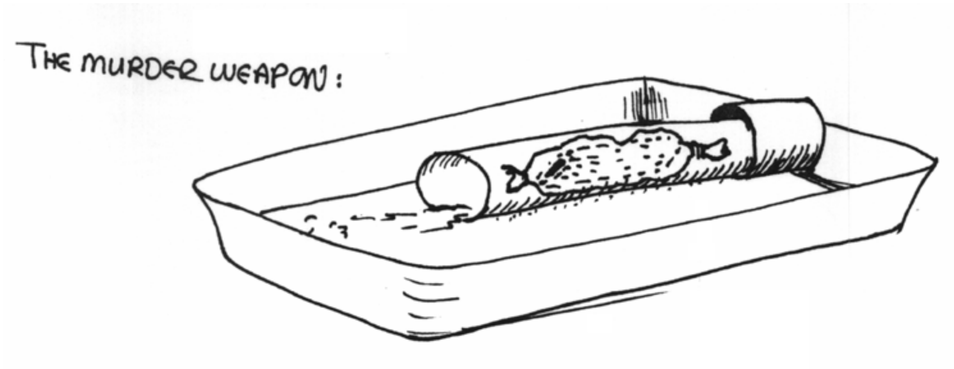
**Watson:** When the constable examined the body at 6:30 AM there were no signs of rigor, so that would put the time of death around 1 or 2 AM?

**Holmes:** I believe so. The doorman admitted three visitors yesterday. All were former students of Prof. Plum and only stayed about half an hour. There was Colonel Mustard at 3 PM, Mrs. White at 7 PM, and finally Miss Scarlet at 11 PM. One of them set the trap that would eventually
kill Plum, the great physiologist.

**Watson:** But what trap? How was he poisoned?

**Holmes:** Use your eyes Watson! See beneath the chair. There is a pan with a whitish precipicate, probably potassium bicarbonate, and I'll wager we'll find traces of cyanide too. Also a test tube containing a cellulose bag, tied at the ends with string. Inside it is a type of semolina called *penne rigate* typical of a province in southern Italy. Any one of Plum's students had access to these materials. Any might have known it was Plums' habit to doze off in that chair after late hours of reading. It's elementary, my dear Watson, osmosis was the murder weapon, combined with enzymes and hydrolysis. Smell this pan.

**Watson:** (a bit hesitant) Are...Are you sure there's no more cyanide? Hmm, smells sort of like baking bread.




**Holmes' Soliloquy**

It's a simple case, and yet, in some ways an instructive one. We can follow the various steps quite clearly, and they show a remarkable subtlety of mind.

First, the murderer prepared a solution of Pasteur's salts, providing all that yeast requires in the elemental nutrients but not sugar. To this the yeast was added, probably in a fairly standard 1% weight by volume ratio. The mixture was stirred and warmed to activate the yeast. After a short while, the murderer transferred a few milliliters into the test tube. This quantity is the first unknown variable.

The next component of the weapon is the cellulose bag. After soaking in water, one end was folded over one centimeter and crinkled together. Then it was clamped shut. Opening the other end, the murderer inserted a *penne rigate* that had been soaked in water. Next was added some of the murderer's own saliva, plus sufficient water so that all air bubbles could be forced up and out of the bag. Then the top was folded over and sealed as the bottom had been.

Now the bag could easily be slipped into the tube and pushed down to meet the yeast suspension, with no bubbles between. Thus began the chain of events that would eventually lead to Plum's untimely demise.

But a final component was necessary; a top layer of water. It had to be enough to allow for osmosis into the bag - so the sides would be snug against the tube - and enough to dissolve the cyanide crystals when the tube tipped over. This unknown quantity is perhaps the most critical variable for our task. Its effect may interact with the quantity of yeast suspension in the bottom in ways that may best be ascertained by an experimental approach.

Now the device was ready to travel. The murderer visited Professor Plum, and was probably offered a spot of tea, as was his custom. No doubt as Plum made the tea, the opportunity arose to place the tray, then the tube in the cap, and finally the cyanide under the chair. The nearsighted Plum would certainly not notice it in the clutter of his study. All that remained was for hydrolysis, diffusion, osmosis, and fermentation to take their inevitable course. The murderer left the scene well before the murder occurred. Leaving us with a mystery: How long before?

**Death by Osmosis Worksheet**

The game's afoot! As principle investigator in this mystery you need to understand how and why this murder weapon works. Holmes cautions us "It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts." (From Sir Arthur Conan Doyle's A Scandal in Bohemia.) But before we collect data we need to get 'up to speed' on the processes involved.

- The temperature in Plum's 'stuffy little study' doesn't deviate from $30^{o}$ C.
- Traces of Pasteur's Salts (elements favorable to yeast growth) are found.
- When Holmes applies Lugol's solution to the penne remains in the bag, it partially stains dark violet, indicating: ______________________________________(1)
  "I deduce this pasta was merely pre-soaked, not cooked" states Holmes.
- Penne Rigate is 84% carbohydrates (4% insoluble fiber, 6% sugars), 14% protein, and 2% lipids. How many mg of starch would be in a 1.3g penne? _______________________(2)
- If 1/4 of this starch were hydrolyzed, how many mg of sugar would result? Remember that each sugar molecule results from the addition of one molecule of ______ (3) from solution. What is the sugar product of this hydrolysis? ______________(4) What is its molecular weight? _____________(5) What % of this was originally part of the starch? _____________(6) Now you can calculate the mg of this product produced by hydrolysis: ____________________(7)
- This hydrolysis proceeds briskly when the enzyme ___________(8) is supplied in saliva. The molecular weight of this enzyme is over 50,000. What molecular structure do enzymes exhibit? ____________________________________________(9)
- The cellulose dialysis tubing allows molecules less than 13,000 m.w. to diffuse through. What will happen as hydrolysis occurs? ___________________________(10)
- In the yeast suspension enzymes are also at work. *Saccharomyces cerevisiae* liberates an enzyme called maltase that produces the monosaccharide _____________.(11) This product diffuses into yeast cells where a complex of enzymes (collectively called *zymase*) catalyze the fermentation of hexoses (in at least 21 separate steps)! Write the balanced equation for this fermentation: ____________________________________________________________(12)
- The gaseous product in this reaction can dissolve partially in water and react to form an acid. Write the equation for this reaction: _________________________(13)
- So far we've covered hydrolysis, diffusion, and fermentation. A third process plays a key role in this 'weapon'. Define osmosis: __________________________(14)
- Neither the Pasteur's salts nor the yeast cells in suspension will have an enduring effect on the dialysis bag. Why not?

_______________________________________________________
_________________________________________________________________(15)

- What molecules in the dialysis bag are likely to have an enduring osmotic effect? What will this effect be? ______________________________________________(16)
- As the gaseous product mentioned above is liberated in tiny bubbles, these will not be able to pass through the dialysis bag because of their surface tension, instead they will be trapped beneath and join together, increasing pressure. How would this process cause the tube to tip over? ____________________________________________________________(17)
- Hydrogen ions from the acid product will readily diffuse up into the top layer of water, causing the pH to _________.(18) When the tube ultimately tips, this solution spills onto the potassium cyanide crystals, causing an exchange reaction and liberating a deadly gas. Draw this reaction: __________________________________________________ (19)

Now that you have some idea how the murder weapon works, there are some unknowns that can only be known by re-enactment. Here we turn to Holmes again: "It is of the highest importance in the art of detection to be able to recognize out of a number of facts which are incidental and which vital. Otherwise your energy and attention must be dissipated instead of concentrated." (From Sir Arthur Conan Doyle's The Regiate.) Consider these variables and a few assumptions:

1. The amount of yeast suspension added to the tube (you may assume a 1% by weight concentration of yeast, stirred and warmed to 35° C in a Pasteur's Salts solution).

2. The penne has soaked at least 10 minutes in water before being placed in the tubing. To this is added 0.5 ml of saliva and enough water to allow all bubbles to be forced from the bag. The penne are somewhat variable in size, about 4.5 cm long on average. Do you think the conditions under which the saliva is collected matter? What conditions would maximize the enzyme concentration? (20)

3. The dialysis bag is cut to 10 cm. Each end is folded over 1 cm and then crinkled lengthwise at least 4 times before being stapled together (It is necessary to use a TOT stapler). Once the penne, saliva, and water have been added, it should be easy to slide the bag down into the tube to meet the yeast suspension, without air trapped beneath the bag.

4. The layer of water on top is necessary for the weapon to work, and 1 to 2 ml would be adequate for this purpose. However this variable may also influence the time required for the weapon to tip over.

Your group should set-up several experimental tubes, deciding which variables to represent and over what range. It is best to follow the rule of one variable when designing experiments. To quantify the effect of each variable absolutely would require careful monitoring of the tubes over time, something you will probably not be able to do. But you should be able to eliminate some possible suspects by checking on your tubes periodically. Assign duties to your members to check your tubes at the times you deem most informative. Be prepared to report back to the class on your procedures, experimental design, and results. Who do you conclude is guilty?

Here's a final quote for contemplation from Holmes: "In solving a problem of this sort, the grand thing is to be able to reason backwards. That is a very useful accomplishment, and a very easy one, but people do not practice it much... There are fifty who can reason synthetically for one who can reason analytically." (From Sir Arthur Conan Doyle's A Study in Scarlet.)

## Notes to the instructor

This lab has several objectives: (1) To review lecture material on biological chemistry, diffusion, and osmosis, (2) To challenge students to think analytically and design and perform an experiment.

Students may work in small groups (4-6) as space and equipment permits.

Ask two students to perform the short play, **Death by Osmosis**. Ask for input from the audience at the appropriate pauses.

Have students do the worksheets in their groups. Most introductory biology texts have information that will be helpful to them.

When the worksheets are done, have someone volunteer to explain to the class how the weapon works. Encourage discussion of ideas from other groups.

Have them regroup and design their experiments. You may want to limit them to 6 tubes/group. This would allow manipulation of the most crucial variables.

You will need to supply pipettes, beakers, Petri dishes, 10 cm long test tubes, stands, 25 mm (1 inch) dialysis tubing, scissors, and forceps for each group.

You may want to have the yeast suspension prepared in advance, or students may make their own. Ask them the difference between a suspension and a solution.

Remind students that strips of dialysis tubing should be soaked in water. I recommend using the TOT stapler to close the bags, however tying the bags with dental floss works well but requires dexterity. Recently, TOT staplers have been modified to use standard staples, which are too wide for the test tubes.

R&F Penne Rigate is made by Ravarino & Freschi, distributed nationwide by Borden Foods.

You want to use test tube caps that are 18 mm in diameter by 25 mm long. Set the tubes in these carefully and far enough apart so if one falls, others aren't knocked over. I put each group's tubes in a separate tray in a large incubator set at $30^{o}$ C.

Obviously, safety is our priority and your reenactments should not include the potassium cyanide. But there's an interesting discussion point here about nitrate poisoning of drinking water (a problem in most agricultural areas). The nitrates change ferrous [$Fe^{2+}$] ions in hemoglobin to ferric [$Fe^{3+}$] ion, forming dark blue methemoglobin (is this an oxidation or a reduction?). This results in a dusky pallor resembling cyanosis. Cyanide poisoning doesn't cause cyanosis, in fact venous blood has more oxyhemoglobin and a redder color than normal. Ironically, a remedy for cyanide poisoning is to administer nitrites or methylene blue to change hemoglobin to methemoglobin, as this reacts with HCN to form non-toxic cyanmethemoglobin.

If you're concerned about bodily fluids in lab, you may substitute porcine pancreatic $\alpha$-amylase for saliva. I have found adding 1.5 ml of a 1 mg% solution (but not additional water in the bag) works almost as fast as saliva. Students may figure this out on their own, but if they chew bread (and rinse) before collecting their saliva, the amylase activity will be greater. For students the act of collecting their own enzyme adds an element of humor to the proceedings and reinforces the role of enzymes in their own bodies.

THE BEST PART of this exercise is that it doesn't really matter who you accuse! Students should check after three hours to implicate or clear Miss Scarlet and after seven hours to implicate or clear Mrs. White. In most re-enactments the tubes will tip in 5-7 hours, implicating Mrs. White. If your setup is different (say you don't have a $30^{o}$ C place to set the tubes), just change the story to suit your conditions.

Pasteur's Salts for 1:9 dilution:

1.0 g potassium dihydrogen phosphate
0.1 g calcium phosphate
5.0 g ammonium tartrate
0.1 g magnesium phosphate
Dissolve the above ingredients in 430 ml deionized or distilled water.
Solution should be autoclaved if long-term storage is desired.

**Worksheet Answers**

1. A dark violet Lugol's solution indicates not all the starch had been hydrolysed.

2. Of the 84% carbohydrates, subtracting the fiber (4%) and sugars (6%) leaves 74% starch. Therefore, 1.3 g penne has 1.3 x .74 = .96 g of starch.

3. Water

4. Maltose

5. I have students calculate the MW of glucose from the formula $C_6H_{12}O_6$: (6 x 12) + (12 x 1) + (6 x 16) = 180. Since maltose is made by combining two glucose and subtracting water, its MW will be: 180 + 180 - 18 = 342.

6. Since maltose is formed in this case by hydrolysis of starch, a water molecule was added for each maltose molecule formed. That means that (342 - 18)/342 = .95 or 95% of each maltose was originally in the starch.

7. From (2) we know .96 g of starch is in a penne. If 1/4 of this was hydrolyzed, as stated in this problem, then .96/4 - .24 g is converted to maltose. But, as we figured previously, the addition of water means that the result will be .24/.95 = .253 g or 253 mg of maltose.

8. Amylase

9. Tertiary structure

10. The amylase and starch cannot diffuse out, but maltose can. There will be a net diffusion of maltose into the solution containing the yeast.

11. Glucose

12. $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$ (ethanol)

13. $CO_2 + H_2) \rightarrow H_2CO_3$ (carbonic acid) $\rightarrow H^+ + HCO_3$- (bicarbonate ion)

14. The diffusion of water through a semi-permeable membrane from a solution with a lower solute concentration into a solution with a higher solute concentration.

15. As solutes, the Pasteur's salts are small enough to diffuse through the bag and reach an equilibrium concentration in both solutions, hence have no enduring osmotic effect. The yeast cells are in suspension, not solution. They are too large to have an osmotic effect.

16. The unhydrolysed starch and amylase remaining as solutes in the bag have an osmotic effect that will draw water into the bag, causing it to swell against the sides of the tube.

17. The bag will be forced upward by the gas pressure beneath. With the mass of the fluids in and above the bag rising, so does the center of gravity, causing the tube to become top heavy.

18. Fall

19. $H^+ + HCO_3 + KCN \rightarrow K^+ + HCO_3$- + HCN (hydrogen cyanide gas).

20. If the student chews bread (or some likewise starchy food) the quantity of amylase in saliva will be greater.