

A low cost model to facilitate students' understanding of electron transfer chain in toxicology lab

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ABSTRACT

Mitochondrial respiration is a complex process which its biochemistry is often poorly understood by undergraduate students when explained in toxicology lectures. The use of experiments to reinforce their knowledge is important, but not always possible because of low teaching budgets. Therefore, a low cost model, made using water, oil, styrofoam and modeling clay, is presented here to simulate the transduction membrane, and the complexes embedded in it. Using this model, students can represent and understand electron flow and proton translocation, the chemiosmotic hypothesis and the effects of inhibitors and uncouplers. Students that have used this model enjoyed studying mitochondrial respiration and learned and understood the biochemistry of transduction membranes as well as lipid and protein interactions, and were well motivated to study the phenomenon in depth by themselves.

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Introduction

In many developing countries like our own the number of students on basic courses such as toxicology is very large, while the availability of materials, equipment and budget for the teaching laboratories is very low. Trying to develop an experiment to demonstrate mitochondrial respiration is difficult when you do not have an oxygen electrode or a refrigerated centrifuge to isolate the mitochondria. Therefore, we decided to design a demonstration exercise whereby the students could visualize the way the respiratory complexes are embedded in the membrane (1), and follow electron transport and proton translocation. We expect the students to understand the concepts of potential difference and the chemiosmotic hypothesis (2), as well as the differences between an inhibitor and an uncoupler (3). These can be learned while the students play and interact with different materials that are inexpensive and easy to obtain. This paper explains one simple model just to show what can be done. However, students could be asked to prepare their own models of the respiratory chain and electron flow or other transduction membranes, and explain them to the group.

Experimental Model

1. Preparation of the transduction membrane
Students need to represent the mitochondrial inner membrane and they can do this by putting water in a glass vessel, and then adding oil to cover the water. They would have then a two-phase system, where; water at the bottom represents the inside of the mitochondrion and the oil phase represents the membrane. (If they were to add more water to represent the inter membrane space or the cytosol, it would, of course, go to the bottom, so they have to imagine there is another water phase on top of the oil.) Another way to represent the membrane is to prepare a three-phase model where the middle phase is made of jelly. The problem with this model is that it does not give us enough freedom to take components in or out of the model because of its rigidity.

To represent the respiratory complexes, styrofoam balls of different sizes could be used. To make sure that the balls or complexes will be immersed in the membrane or oil phase and will not float on it, they can be filled with modeling clay. At the same time the modeling clay represents the inner part of the enzymes where the prosthetic groups are, while the lipophilic part of the enzyme, which is in contact with the membrane, is represented by the styrofoam balls (Fig. 1).

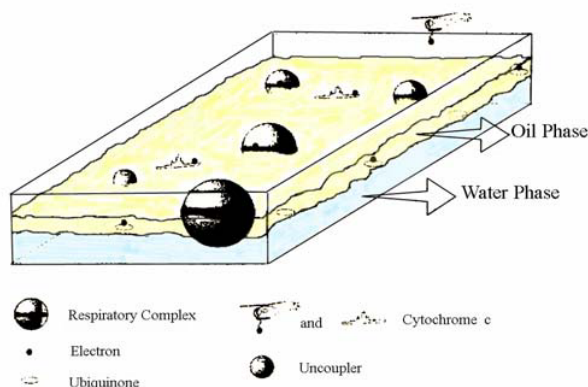


FIGURE. 1. Schematic representation of the mitochondrial innermembrane. The model is made in a glass vessel where the water represents the mitochondrial matrix and the oil represents the membrane. The respiratory complexes are represented by styrofoam balls filled with modeling clay, and the electrons by smaller balls of the same material. Electron carriers such as ubiquinone and cytochrome c, are represented by lentils and paper boats or helicopters, respectively, and an uncoupler is represented by a Styrofoam ball with a hole through it.

The first part of the exercise is the preparation of the respiratory membrane and the comparison of the movement and behavior of the styrofoam balls with the respiratory complexes. Students will observe their independent diffusion and collision in agreement with the random collision model (4), by moving the glass vessel or gently shaking the oil phase.

2. Electron flow, prosthetic groups and electron carriers

As mentioned, the prosthetic groups are represented by the modeling clay inside the balls, and the electrons can be also represented by very small balls of modeling clay which can easily be attached to the prosthetic groups. The electrons have to move from one membrane complex to the other without being in touch with the oil. At this point the students are faced with the problem of electron transport. How can these electrons go from one complex to the other if they can not move in the oil phase because of their charge? One way to solve this problem is to use an electron carrier that would be able to float in the oil and go

from one complex to the other (a lentil or a bean can be used to represent ubiquinone, a little piece of paper, and even a little paper boat or a paper helicopter can represent the cytochrome c, Fig. 1). When the students solved these problems using their knowledge of the respiratory chain and their imagination, they can represent the whole electron flow from NADH to oxygen. (The NADH and the oxygen could be represented with little pieces of colored paper or modeling clay balls bigger than the electrons and of a different color.) Even if the sizes of the complexes and the carriers are not proportional to the real components they can give a good idea of the transduction membrane.

3. Inhibitors

Since inhibitors are molecules that attach to specific parts of the respiratory complexes and interrupt electron flow, their behavior can be represented by wrapping the modeling clay prosthetic groups parts of the complexes with masking tape, so that electrons cannot attach to them. Students must then try to carry out the electron flow and visualize if there is still any oxygen consumption or not. Oxygen consumption occurs when electrons can move through all the complexes and arrive at oxygen. If the electron's pathway is blocked or the electron itself is blocked and does not arrive at the oxygen, there is therefore no oxygen consumption. To understand the different inhibitors and their sites of action, students can also wrap complex I and start the electron flow from complex II by attaching the electrons directly to that complex, and discover if there is oxygen consumption or not. The same can be done by wrapping complexes III and IV, and starting the flow from different sites.

4. Chemiosmotic hypothesis

Students can represent electron flow using this model but they can also characterize proton translocation by using modeling clay of a different color to represent the protons. However, it is not possible to move protons from the inside of the mitochondria (water phase) to the inter membrane space (which is represented by the air on top of the oil), and to maintain them there. To solve this problem, a wax layer can be made in order to isolate both phases and to create a differential potential of protons. This layer can be made by setting

drops of wax from a candle gently over the oil. When the wax cools it will provide a firm support for the modeling clay protons. One technical problem is that the wax layer could immobilize the respiratory complexes in their places, but if students perceive this as a technical problem, it will not really interfere with their understanding. The F_0F_1 ATP synthase (5) or an uncoupling agent can be represented by the same Styrofoam balls with a hole that runs through from side to side. Protons can then be dropped in from the outside to the inside. Another way to represent uncoupling is just to make a hole on the wax layer and drop the protons through it.

Study questions

- 1) What is an oxidation-reduction potential?
- 2) What are the complexes that form the mitochondrial respiratory chain?
- 3) What is a prosthetic group, and which prosthetic groups comprise the mitochondrial respiratory chain?
- 4) What is an electrochemical potential?
- 5) What is a coupling site?
- 6) What is the effect of an inhibitor and of an uncoupler?
- 7) Explain in your own words, with an example, what the chemiosmotic hypothesis is about?
- 8) Where does the ATP synthesis take place and where does the necessary energy come from?
- 9) Carry out a bibliographical (literature) search to find out about organisms in which the respiration phenomenon takes place.
- 10) Carry out a bibliographical search to find out about other phenomena that take place in transduction membranes.

Results and Discussion

This laboratory class experiment has been designed and performed by the author in Faculty of Veterinary Medicine, Azad University, Tehran, Iran since last year. It was very useful in enabling students to understand the concept of transduction membranes and the chemiosmotic hypothesis. They seem to visualize the phenomenon as an interactive and dynamic event rather than as a list of reactions written on the blackboard. An important thing we realized while doing this experiment, was that students did not understand the concepts of protein and lipid structure and their interactions, especially in membranes, and the present exercise gave students a new vision of protein and lipid biochemistry. Students were very excited with this experiment because they felt they were learning while they played, and that motivated them to do literature searching and to read more deeply about the subject.

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