Lodz University of Technology
Institute of Materials Science and Engineering

Laboratory 3

Estimation of transport across the cell membrane

Instruction for the Laboratory of Biophysics

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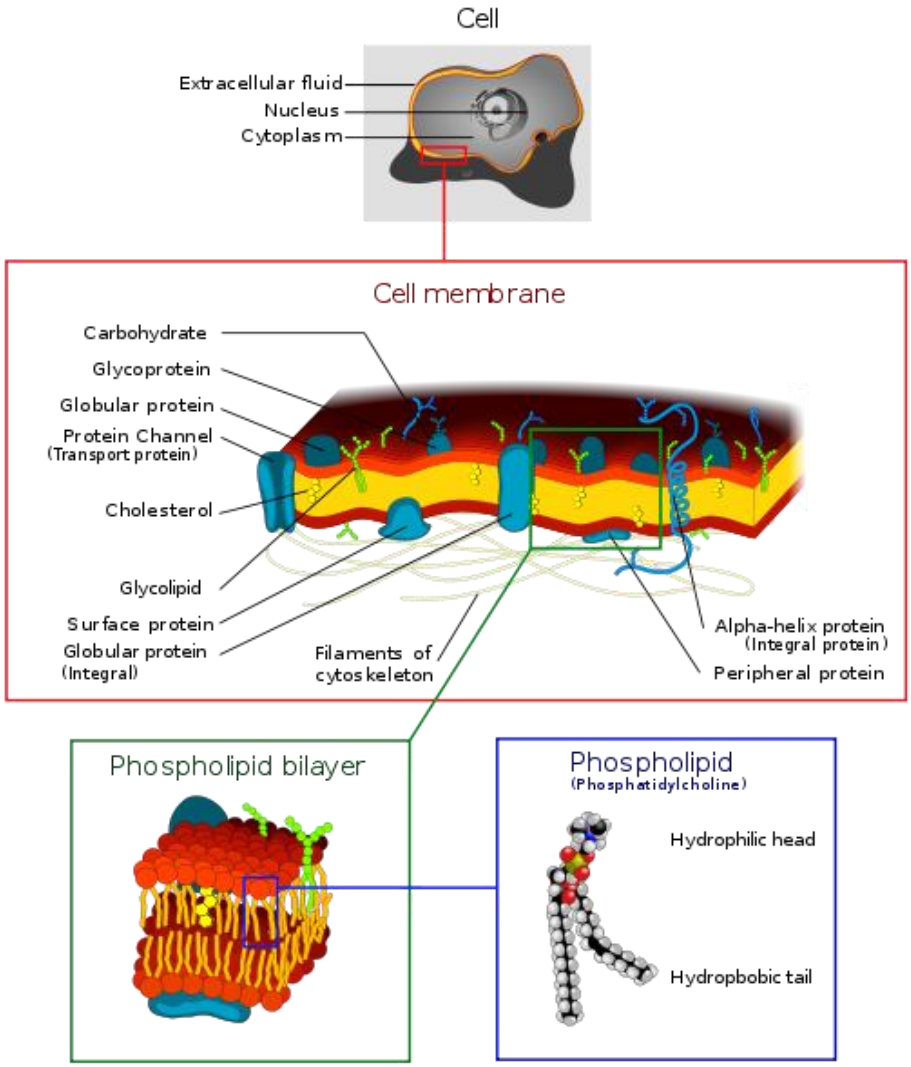
I. THEORETICAL INTRODUCTION

The **cell membrane** (also called the **plasma membrane**, **plasmalemma**, or "phospholipid bilayer") is a selectively permeable lipid bilayer found in all cells. It contains a wide variety of biological molecules, primarily proteins and lipids, which are involved in a vast array of cellular processes such as cell adhesion, ion channel conductance and cell signaling. The plasma membrane also serves as the attachment point for both the intracellular cytoskeleton and, if present, the extracellular cell wall.



(Source: the Internet)

Membrane transport is the moving of biochemicals and other atomic or molecular substances across biological membranes.

Usually, two types are distinguished: Active transport requires chemical energy, while passive transport does not.

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Membrane transport refers to carrying of substances across the cell membrane. It can be divided into two types: Membrane transport protein

1. Against concentration gradient and requiring energy for transport - called active transport

2. Along the concentration gradient and does not require energy for transport- called passive transport

Passive transport can be of two types:

1. Simple diffusion- crossing over of ions and other chemical substances across the cell membrane that is dependent on concentration gradient- substances diffuse across the cell membrane from higher concentration to lower concentration.

2. Facilitated transport- usually using a carrier that binds to the substance and carries it across the cell membrane without involving energy expenditure. For example, transport of iron by transferrin across the cell membrane.

Based on their specificity for carried substances, a number of transporters have been identified, like ATP-binding cassette transporter, Glutamate transporter, Neurotransmitter transporter, Glucose transporter, Dopamine transporters among others. These transporters can be grouped into two groups:(1) ATP Binding Cassette Transporter (ABC transporter) and (2) Solute Carrier Family.

Transporters are proteins that may be situated on the membrane surface or may be present in the cell cytoplasm. The later are activated by binding of the ligand to its receptors to reach the membrane surface for binding the substance to be transported.

Facilitated diffusion is the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane. Many large molecules, such as glucose, are insoluble in lipids and too large to fit through the membrane pores. Therefore, it will

bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane. Bear in mind, however, that facilitated diffusion is a passive process, and the solutes still move down the concentration gradient.

Active transport directly uses energy to transport molecules across a membrane.

Most of the enzymes that perform this type of transport are transmembrane ATPases. A primary ATPase universal to all cellular life is the sodium-potassium pump, which helps maintain the cell potential. Other sources of energy for Primary active transport are redox energy and photon energy (light). An example of primary active transport using Redox energy is the mitochondrial electron transport chain that uses the reduction energy of NADH to move protons across the inner mitochondrial membrane against their concentration gradient. An

example of primary active transport using light energy are the proteins involved in photosynthesis that use the energy of photons to create a proton gradient across the thylakoid membrane and also to create reduction power in the form of NADPH.

II. EXPERIMENTAL PART

A) Preparation of samples - estimation of yeast cytocrit

1. Precisely mix suspension of yeast and add 1000µl to each 4 eppendorf tube, next centrifuge by 1 min, 10 000 rpm in room temperature.
2. The supernatant pour out from eppendorf tube, add 1000µl phosphate buffer and mix a suspension of cell. Next centrifuge by 1 min, 10 000 rpm in room temperature.
3. The operations from point A 2 repeat twice
4. Remove supernatant very precisely and yeast cell suspend in 100 µl buffer.
5. After consultation with instructor fill up capillars for ¾ capacity (one capillar for one tube)
6. In the presence of instructor lock up capillars by used the burner and centrifuge by 1 min, 800 rpm.
7. For each capillar estimate cytocrit – 100% is volume of whole cappilary, and an area of cell suspension gives % of cytocrit. Next calculate average cytocrit.

B) Preparation of samples – cell purification

1. 1000µl yeast suspension gives a cytocrit estimated in part A. Using proportion calculate a volume of yeast which gives 5% cytocrit.
2. For 10 Eppendorf tube add a volume of yeast estimated in part B1. Next centrifuge by 1 min, 10 000 rpm in room temperature.
3. The supernatant pour out from eppendorf tube, add 1000µl phosphate buffer and mix a suspension of cell. Next centrifuge by 1 min, 10 000 rpm in room temperature.
4. The operations from point B3 repeat twice times
5. The supernatant remove very carefully and yeast cell suspend in 1000 µl buffer.

C) Estimation of transport across the cell membrane

1. Turn on the fluorometer.
2. For 8 samples prepared in part B add 10µl fluorescence dye, mix intensely and turn on a stopwatch.
3. Take 2 tubes, centrifuge by 1min (10 000 rpm), remove supernatant, add 1000 µl buffer and stir.
4. Fluorometer TKO 100 reset „for air”,
5. After 5min fill up a fluorescent cuvette, and add to a cuvette additional 1000µl buffer .
6. Read a result for each tubes from pair and results put in table
7. The operations from points 3, 4, 5 repeat after 10 min, 15 min and 20 min, each time take new pair of tubes. Results put in table 1. Next repeat practice for one pair without added dye – this is result for time 0 minutes.

Tab.1. **Estimation of transport across the cell membrane**

Time (min)	Result	Result	Mean
0			
5			
10			
15			
20			

III. THE REPORT

The report should include:

1. A short theoretical introduction.
2. Tables with results and graphical chart of the following - relation between fluorescence and time
3. Summary.

IV. ISSUES TO STUDY

1. Construction of the cell membrane.
2. Characterize of the transport types through the membrane.

V. REFERENCES

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