MIX-2010w-11T2 - Permeability of RBC membrane – Introduction

3rd year Laboratory course in Animal Biology

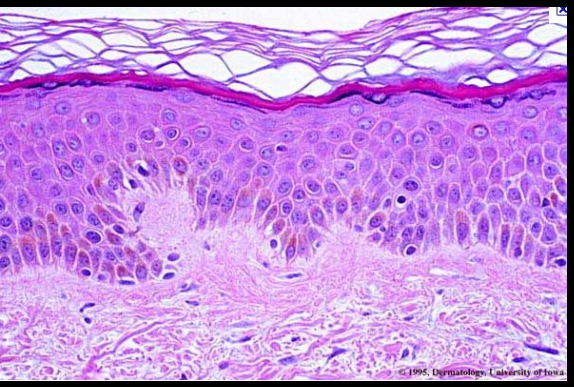
Very first lab!

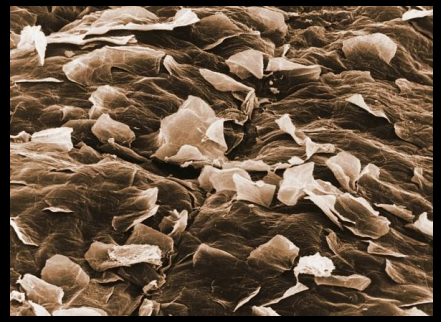
Concepts: recall from their 1st and 2nd year – not covered in 3rd lecture.

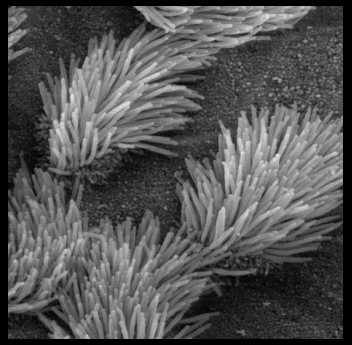
Goals: familiarise students with lab set-up!

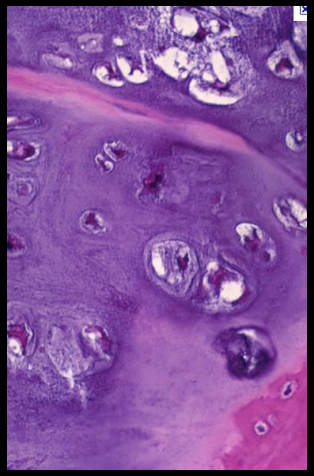
Protocol and lab techniques are kept very simple.

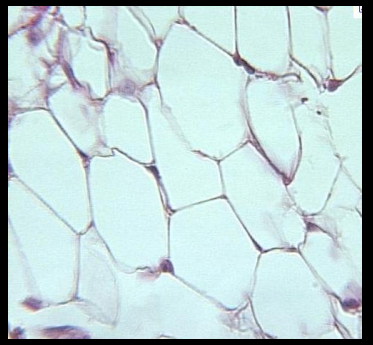
Permeability of RBC membrane – Introduction

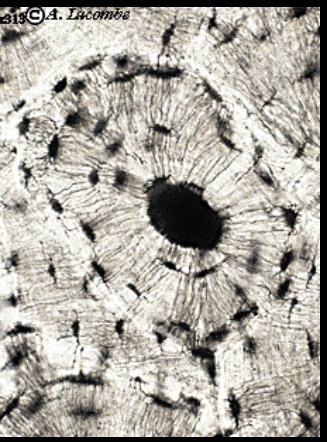


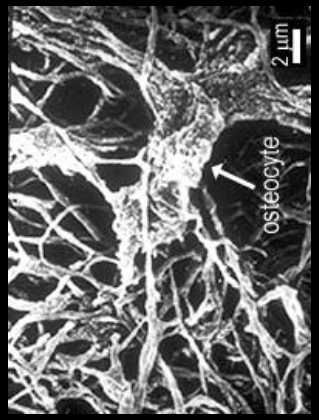


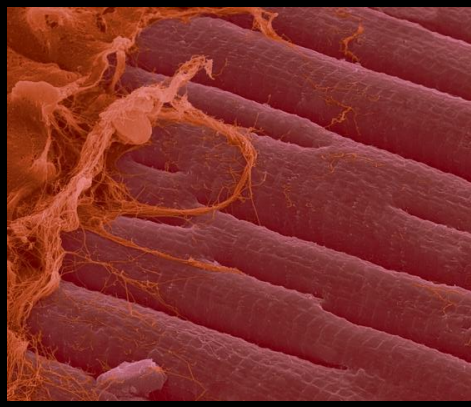


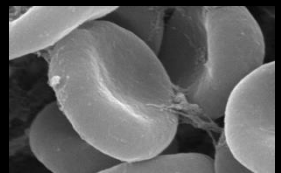












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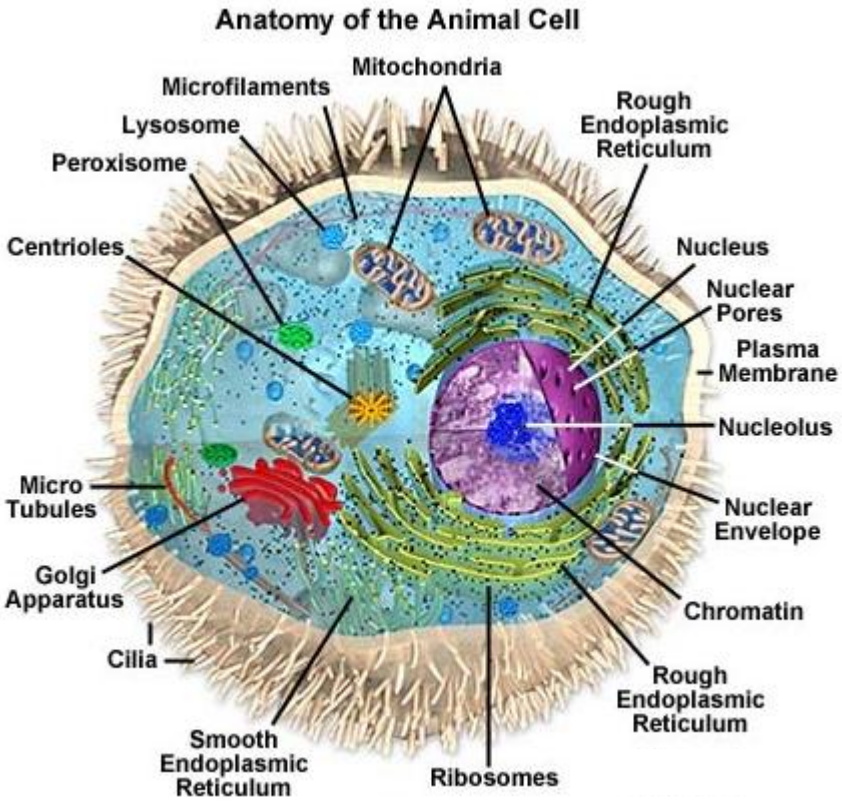

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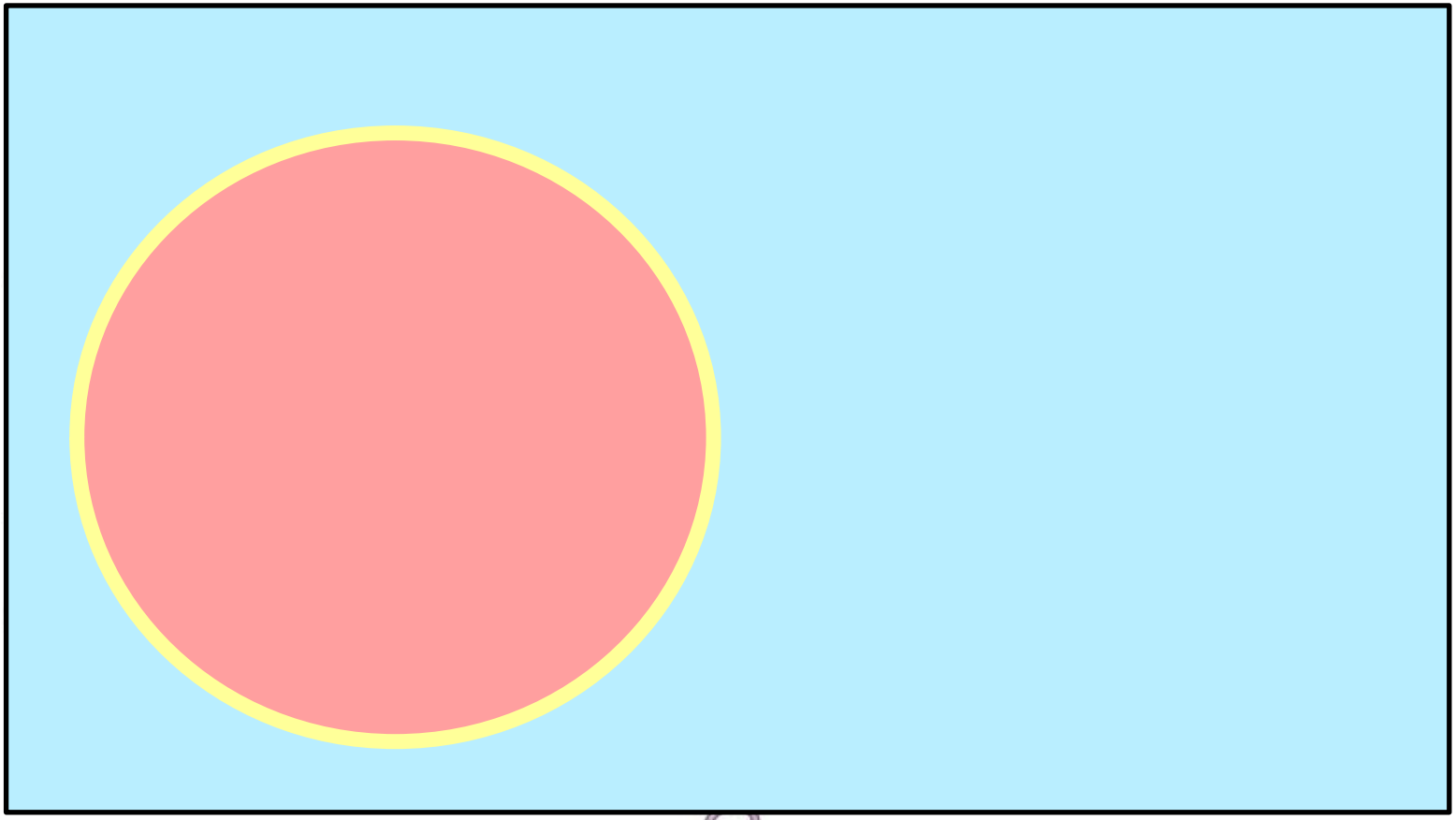

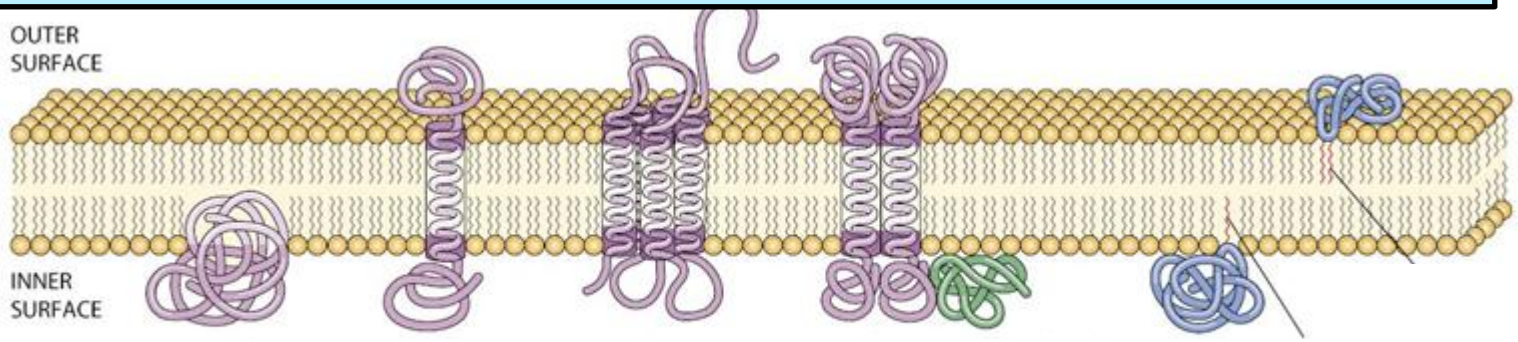

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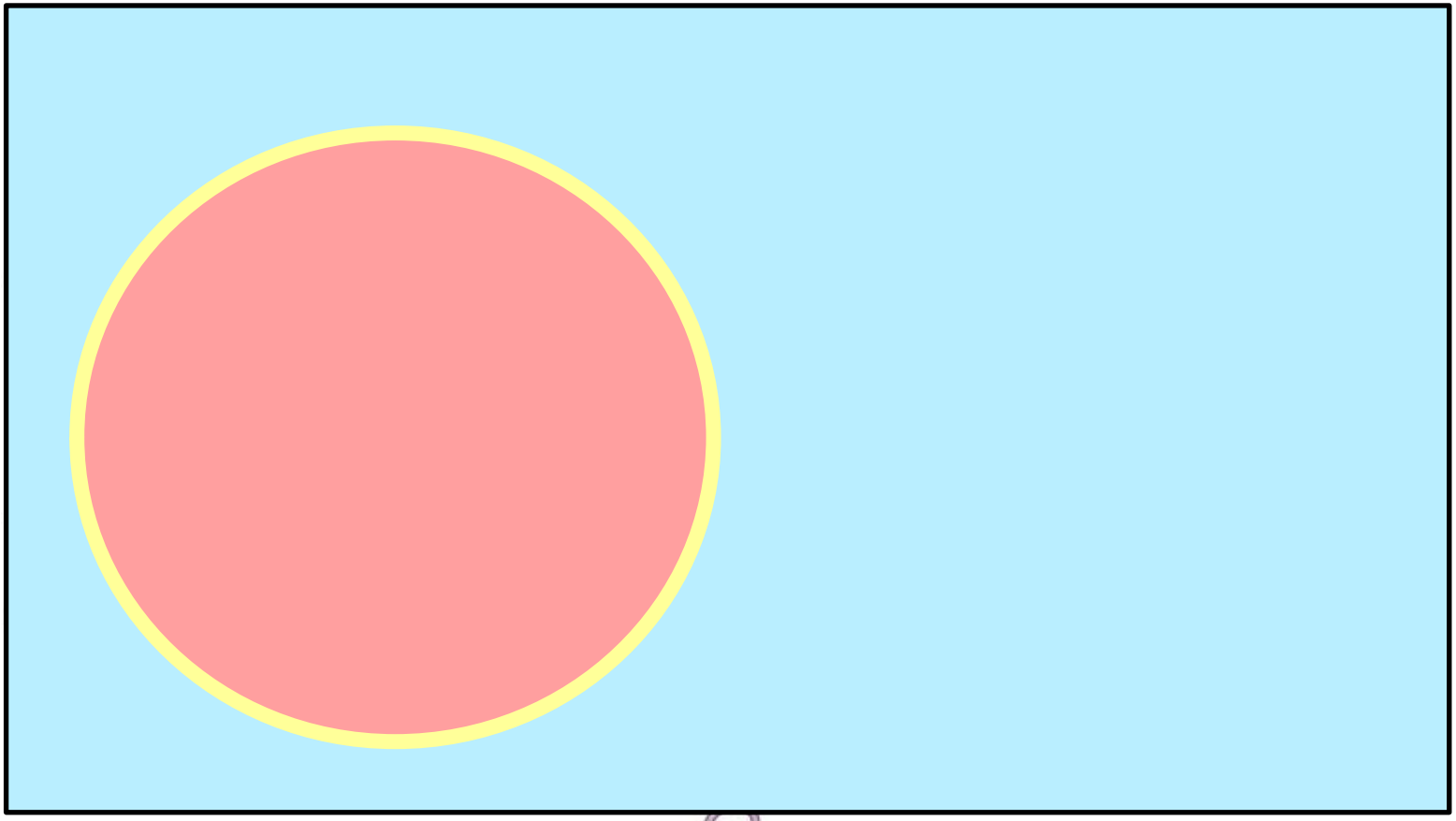
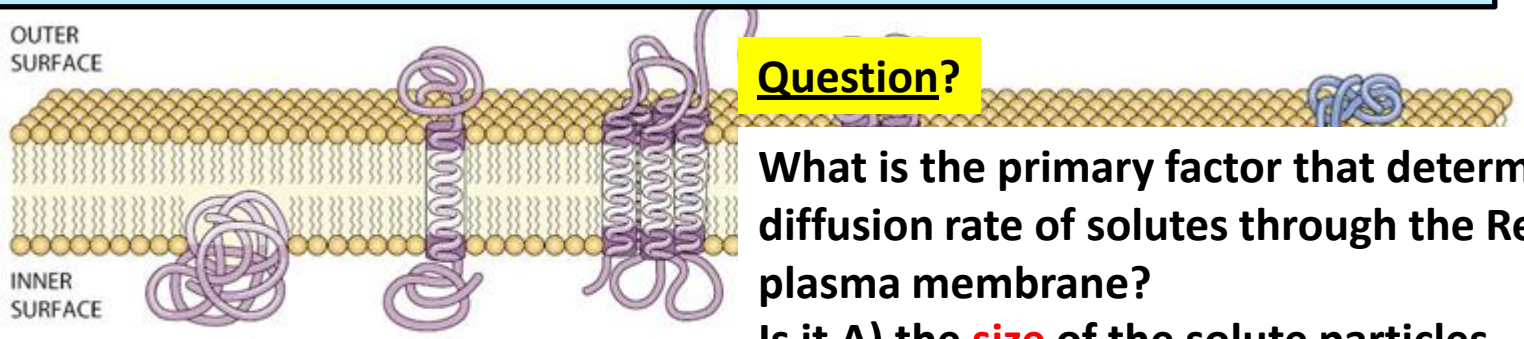




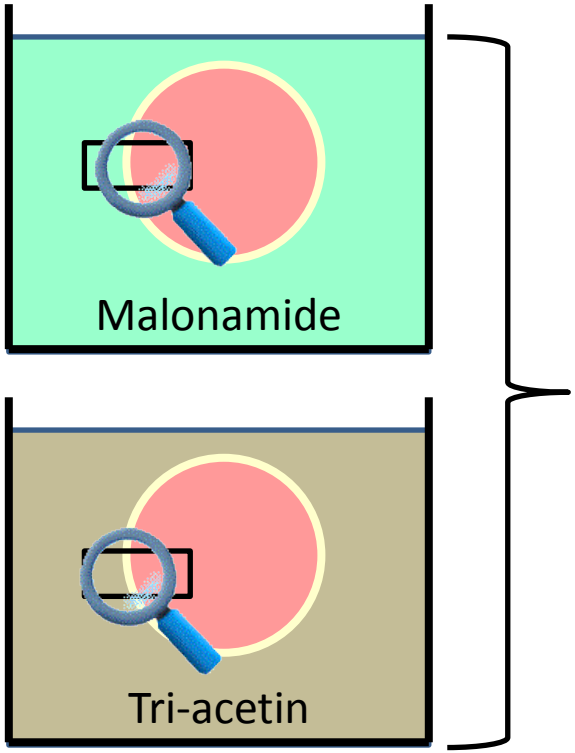
Question?

What is the primary factor that determines the diffusion rate of solutes through the Red Blood cell plasma membrane?

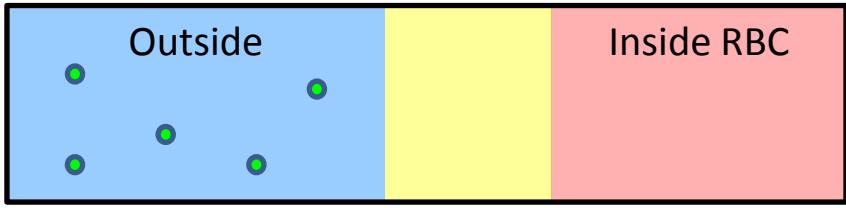
Is it A) the size of the solute particles

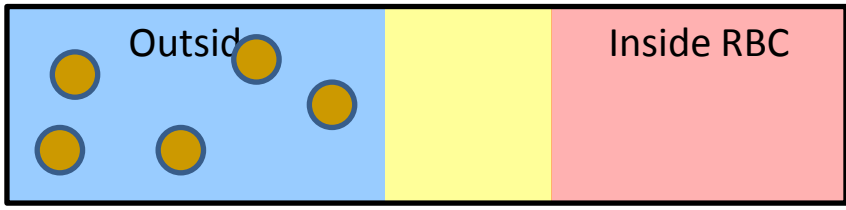
or is it B) their solubility in lipids?

Permeability of RBC membrane – Protocol



≠ lipid solubility
≠ size





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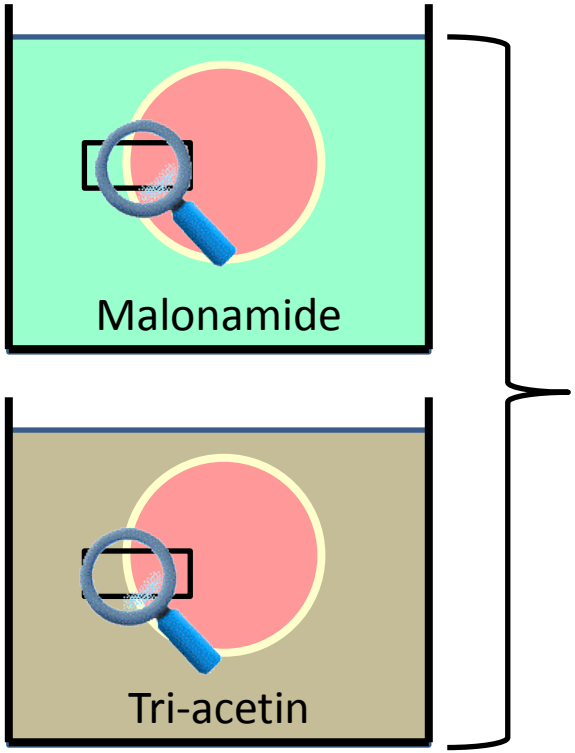
Permeability of RBC membrane – Protocol



≠ lipid solubility

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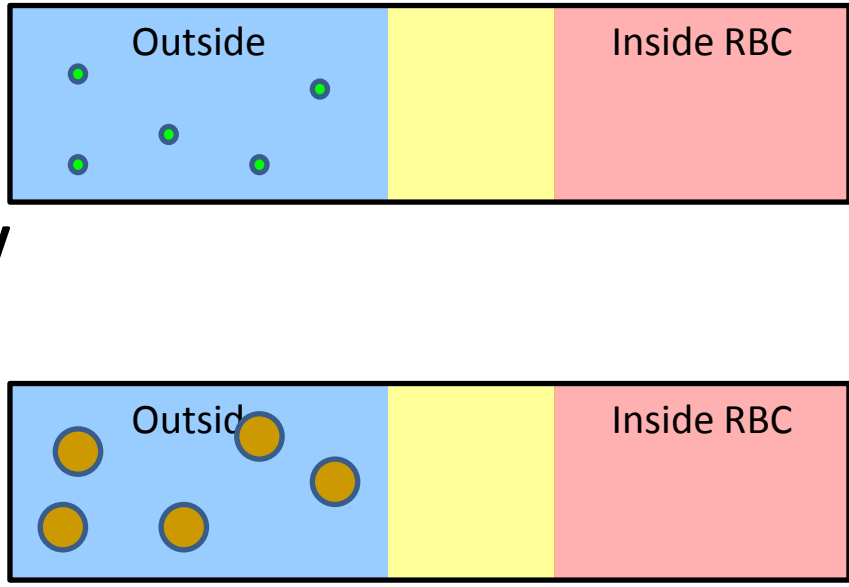


We wait for the cells to blow up (= hemolysis)!

Permeability of RBC membrane – Protocol

Solutes	MW (g)	PC
Glycerol	92	0.00007
Glucose	180	0.00003
Sucrose	342	0.00003
Ethanol	42	0.032
Diacetin	176	0.071
Triacetin	218	0.44
Malonamide	102	0.00008
Lactamide	89	0.00075
Dimethyl urea	88	0.0023
Methyl Alcohol	32	0.0097

→ **hemolysis time is a good estimation of the diffusion rate**

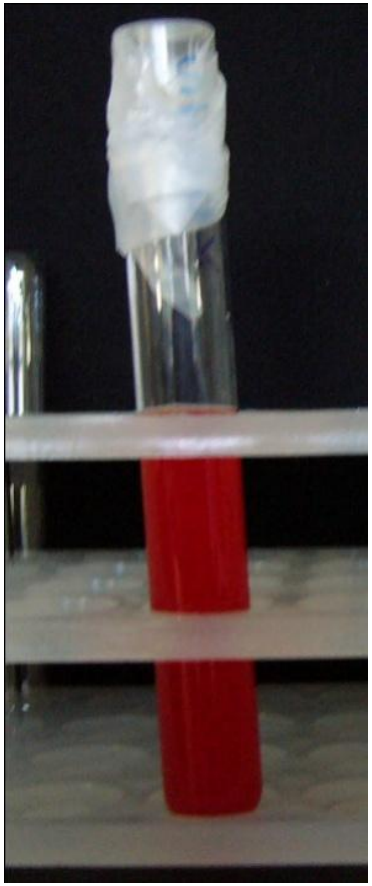
→ **molecular size (diameter) roughly parallels molecular weight.**

→ **the partition coefficient of a solute is a measure of its relative solubility in water and lipids.**

$$\text{Partition coefficient} = \frac{\text{solute concentration in oil}}{\text{solute concentration in water}}$$

Permeability of RBC membrane – Data? Data analysis?

Permeability of RBC membrane – Methodology - Stock blood suspension



Permeability of RBC membrane – Methodology – Hemolysis time

Steps to test for hemolysis

1- Take a test tube;

2- Add 5 ml of the solution to be tested;

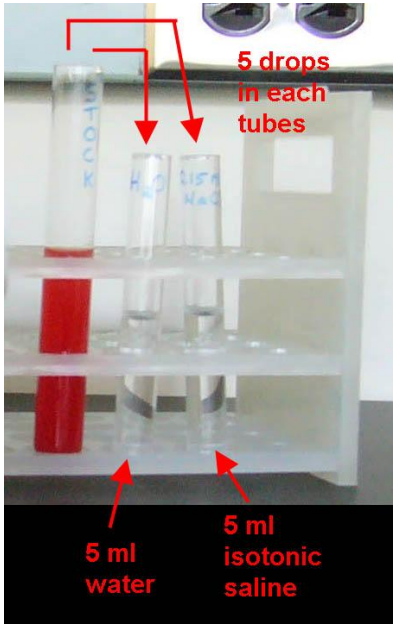
3- Add 5 drops of stock blood suspension;

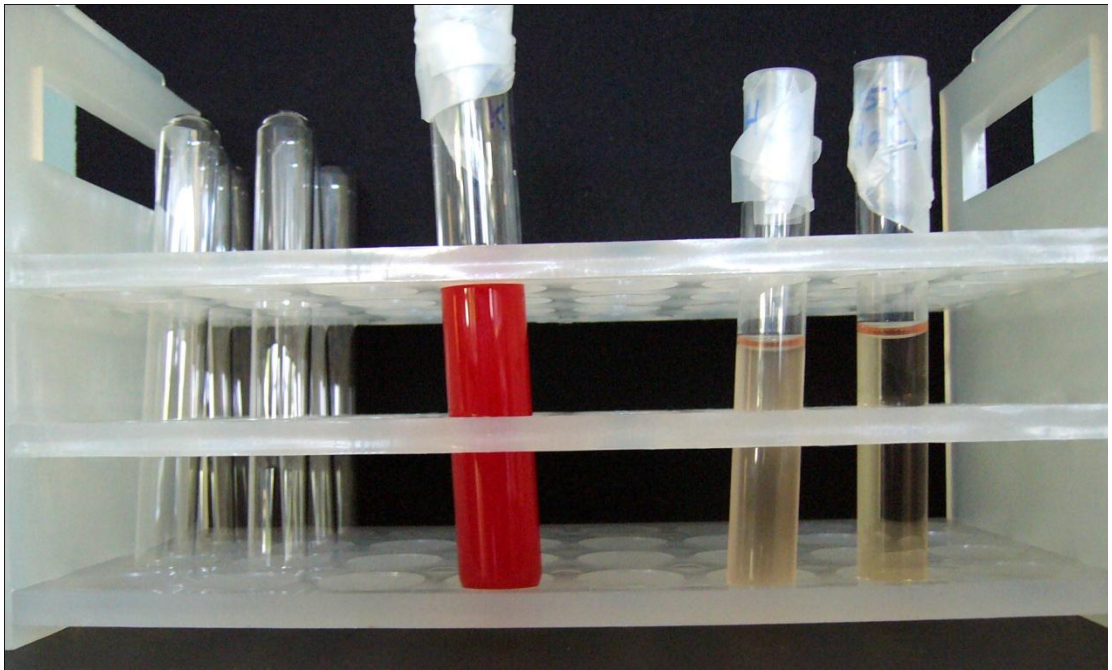
4- As the fifth drop of blood reaches the solution, start the timer and mix gently;

5- Wait for ALL the red blood cells to be hemolysed.

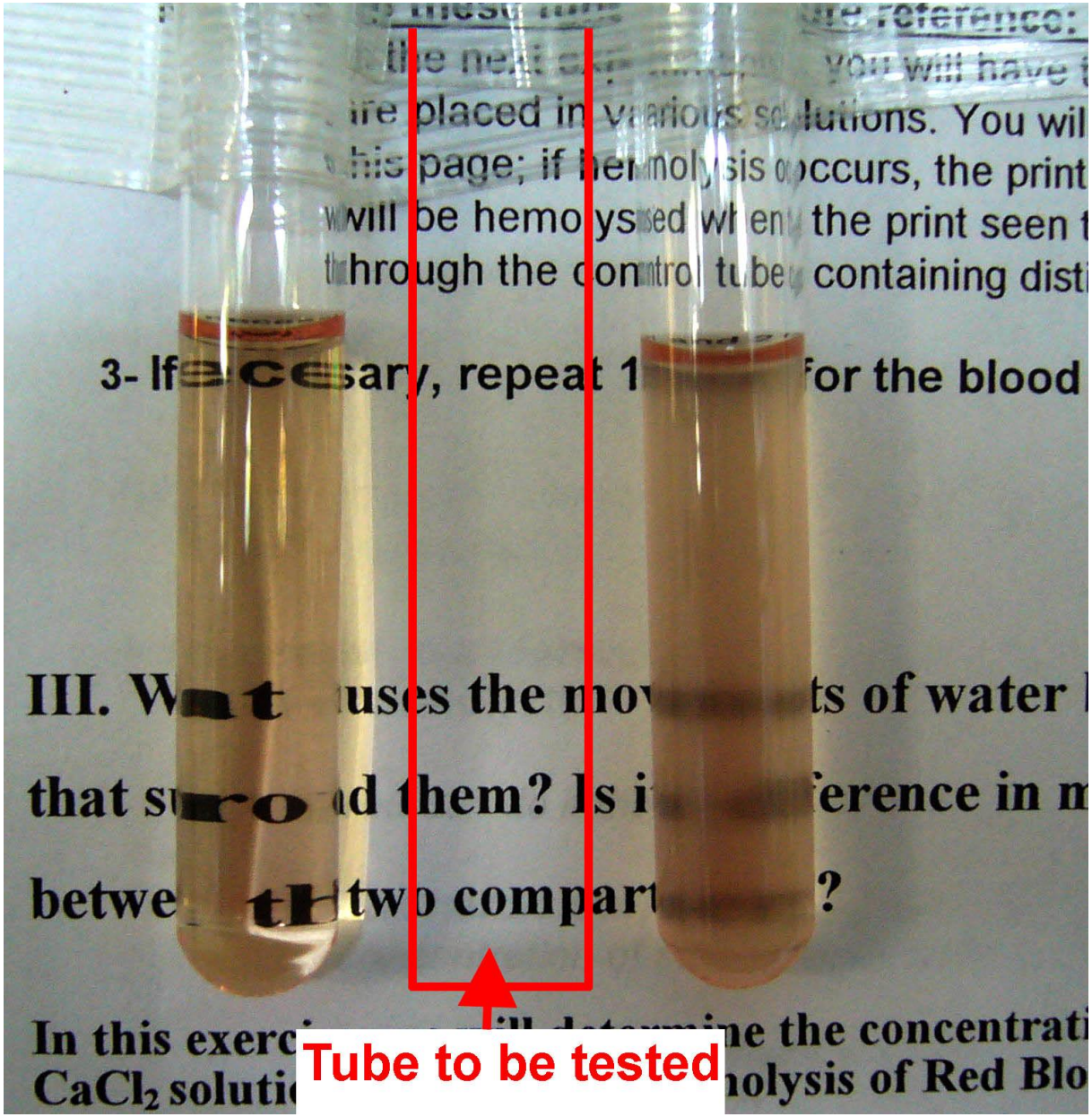
6- Record the time in the excel file.

Permeability of RBC membrane – Methodology – Reference tubes





Permeability of RBC membrane – Methodology – Reference tubes

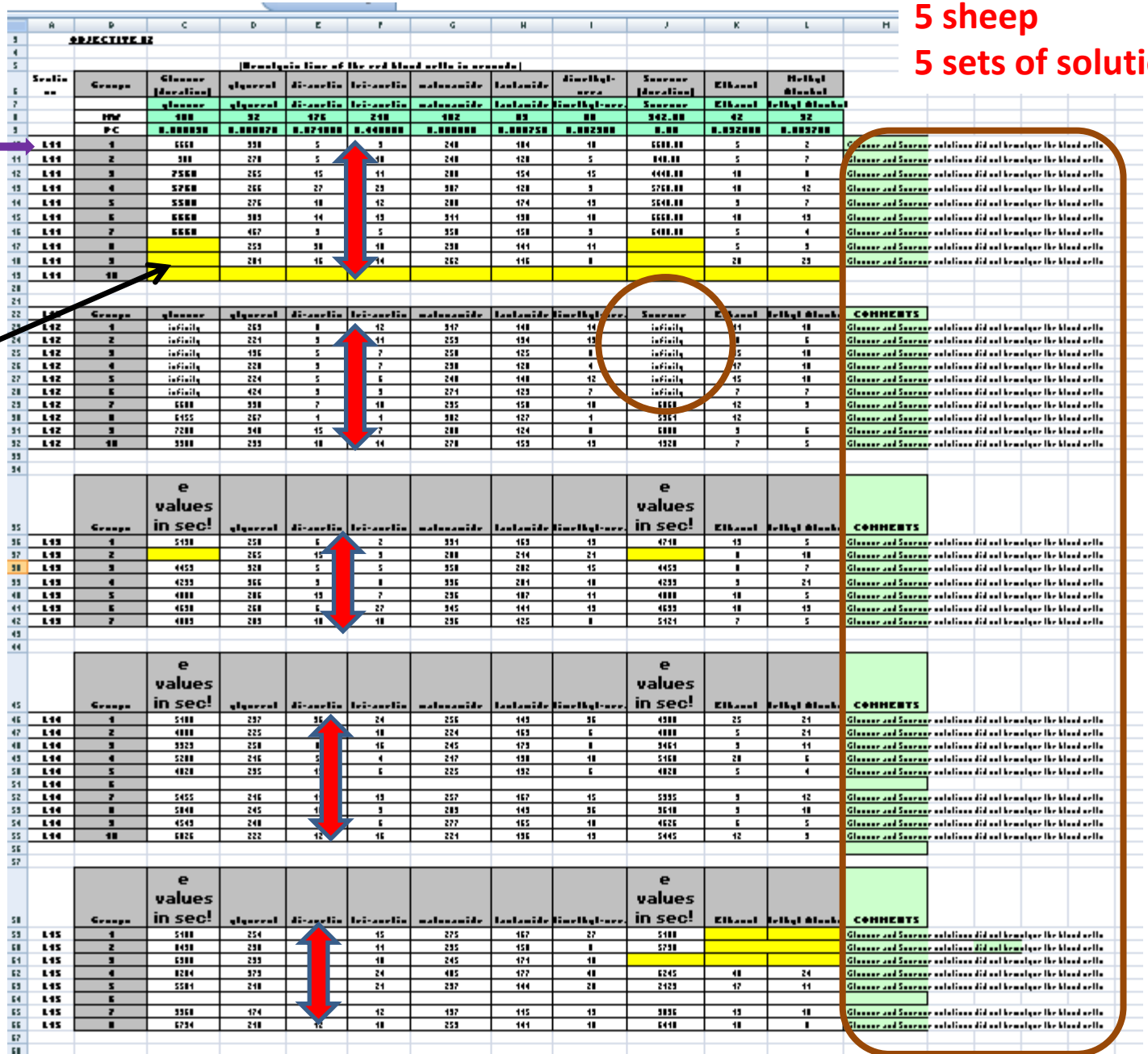


Permeability of RBC membrane – Data collected

5 sheep
5 sets of solutions

Set of data gathered by one pair of students

Yellow: missing data



Permeability of RBC membrane – Data collected

OBJECTIVE #2

(Hemolysis time of the red blood cells in seconds)

Sections	Groups	Glucose (duration)	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl-urea	Sucrose (duration)	Ethanol	Methyl Alcohol	
		glucose	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl-urea	Sucrose	Ethanol	Methyl Alcohol	
	MW	180	92	176	218	102	89	88	342.00	42	32	
	PC	0.000030	0.000070	0.071000	0.440000	0.000080	0.000750	0.002300	0.00003	0.032000	0.009700	
L11	1	6660	330	5	3	240	104	10	6600.00	5	2	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	2	900	270	5	10	240	120	5	840.00	5	7	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	3	**7560**	265	15	11	280	154	15	4440.00	10	8	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	4	**5760**	266	27	23	307	120	9	5760.00	10	12	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	5	**5580**	276	10	12	280	174	13	5640.00	9	7	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	6	**6660**	303	14	19	311	138	18	6660.00	10	13	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	7	**6660**	467	3	5	350	150	3	6480.00	5	4	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	8		253	30	10	290	141	11		5	3	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	9		281	16	14	262	116	8		28	23	Glucose and Sucrose solutions did not hemolyse the blood cells
L11	10											

L12	Groups	glucose	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl-urea	Sucrose	Ethanol	Methyl Alcoho	COMMENTS
L12	1	infinity	263	8	12	317	148	14	infinity	11	10	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	2	infinity	221	9	11	259	134	13	infinity	8	6	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	3	infinity	196	5	7	258	125	8	infinity	15	10	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	4	infinity	220	9	7	238	128	4	infinity	17	10	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	5	infinity	224	5	6	248	140	12	infinity	15	10	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	6	infinity	424	3	3	271	129	7	infinity	7	7	Glucose and Sucrose solutions did not hemolyse the blood cells
L12	7	6600	390	7	10	295	150	10	6060	12	9	Glucose and Sucrose solutions did not hemolyse the blood cells

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