

3rd year Laboratory course in Animal Biology

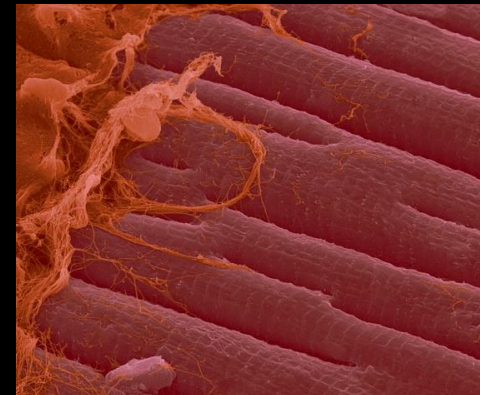
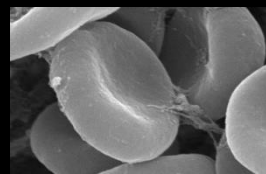
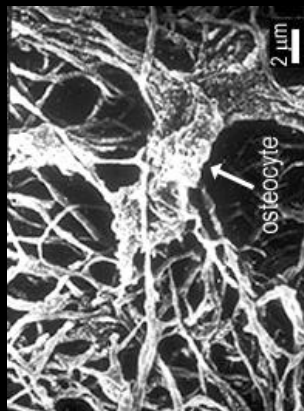
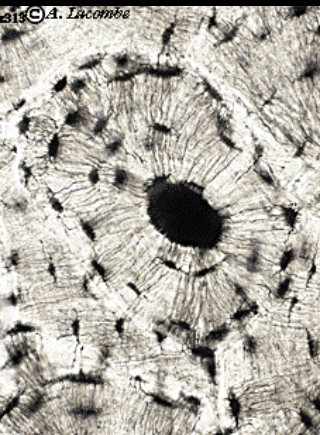
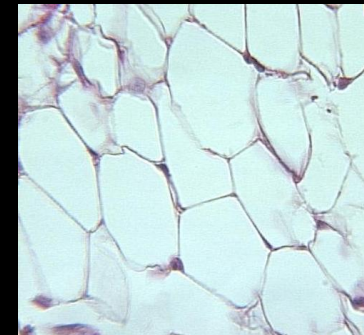
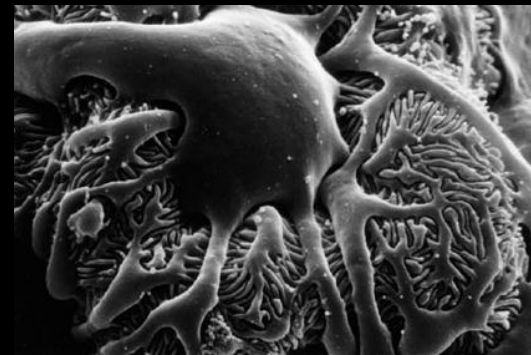
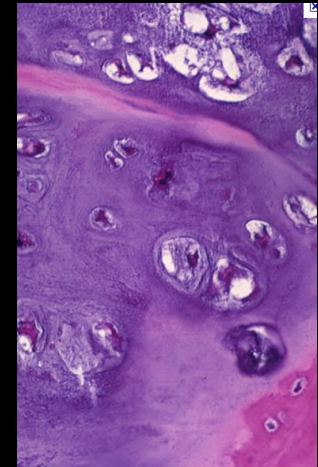
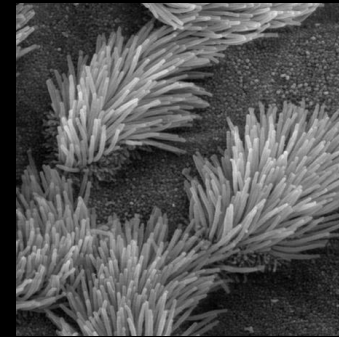
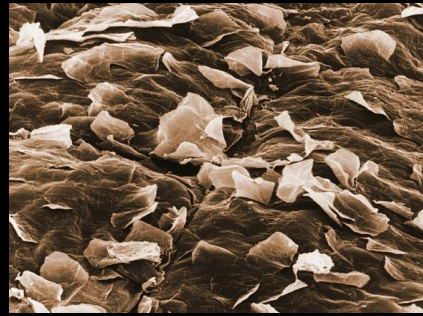
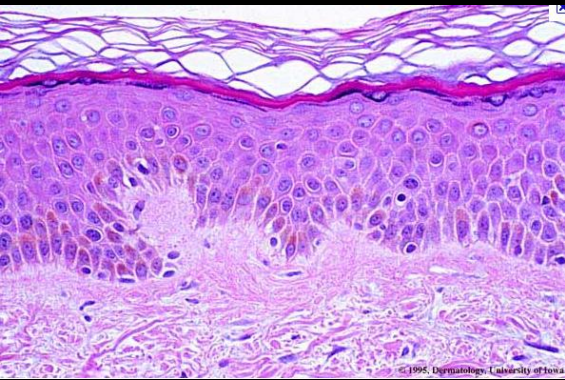
Very first lab!

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

Goals: familiarise students with lab set-up!

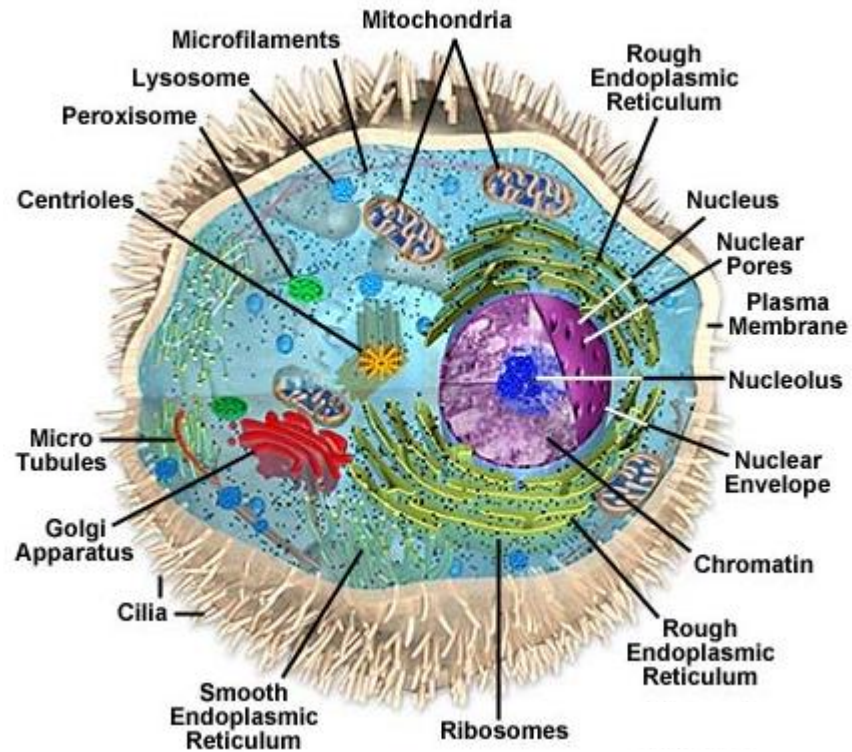
Protocol and lab techniques are kept very simple.

Permeability of RBC membrane - Introduction

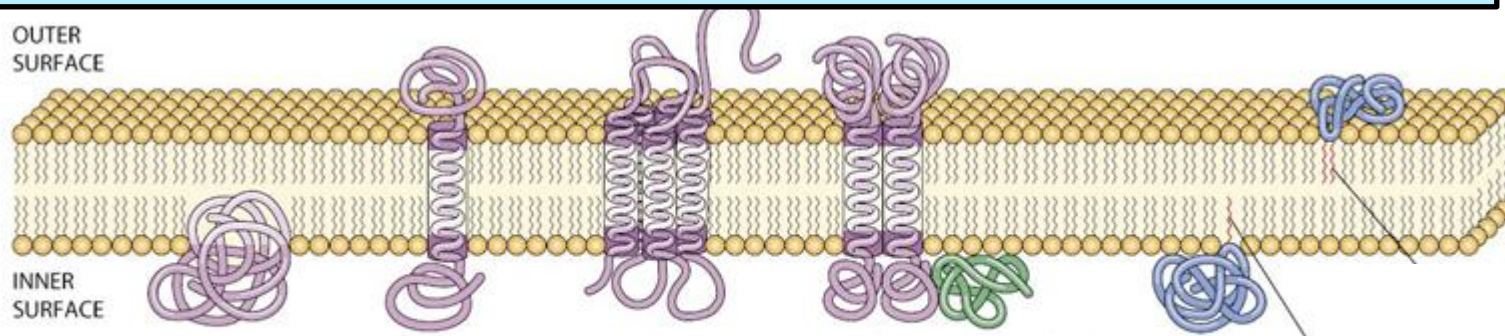
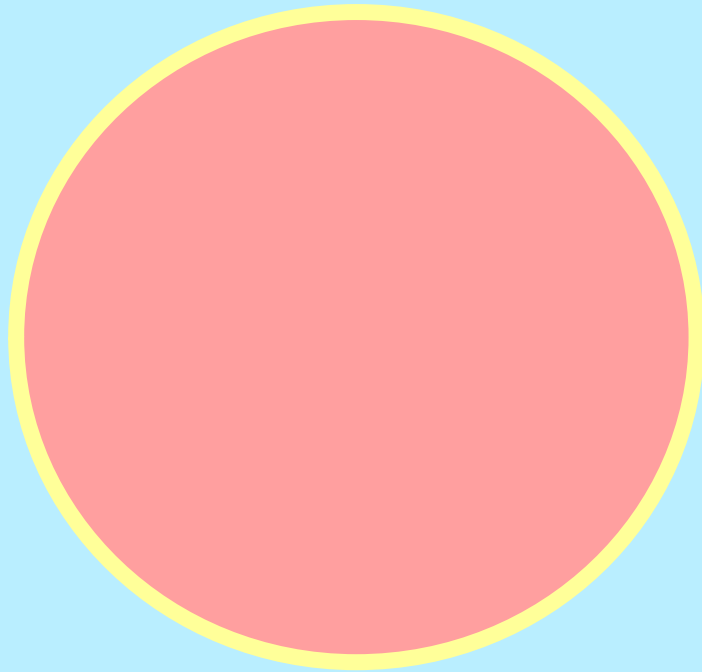


Permeability of RBC membrane - Introduction

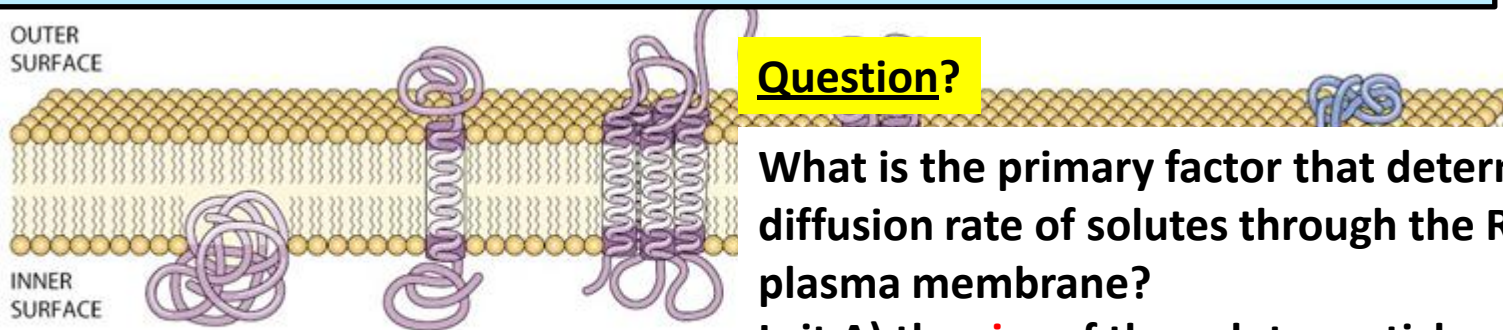
Anatomy of the Animal Cell



Permeability of RBC membrane - Introduction



Permeability of RBC membrane - Introduction

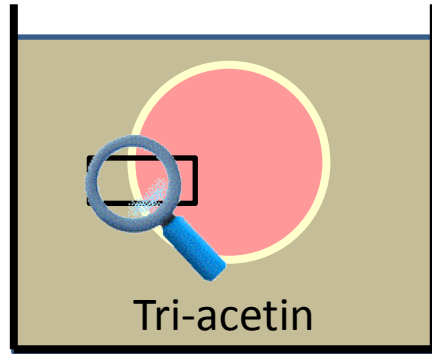
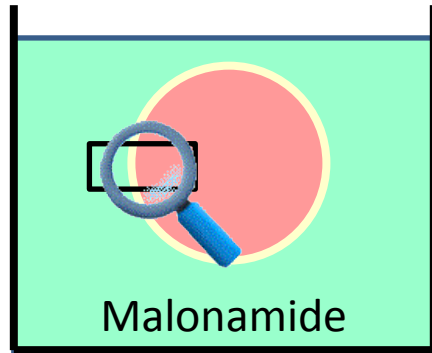


Question?

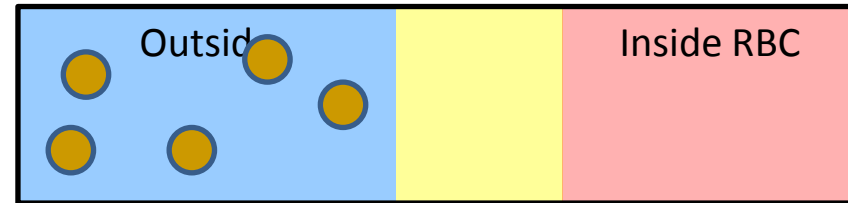
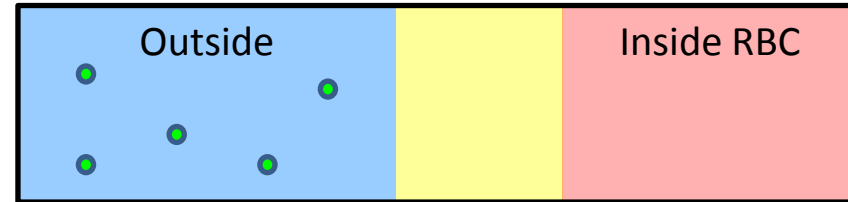
What is the primary factor that determines the diffusion rate of solutes through the Red Blood cell plasma membrane?

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

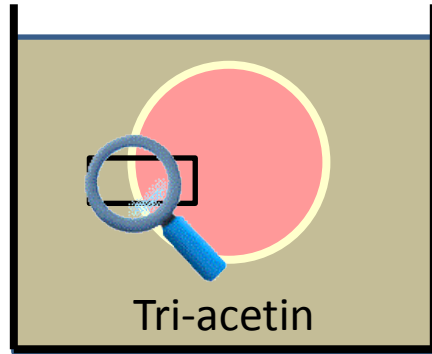
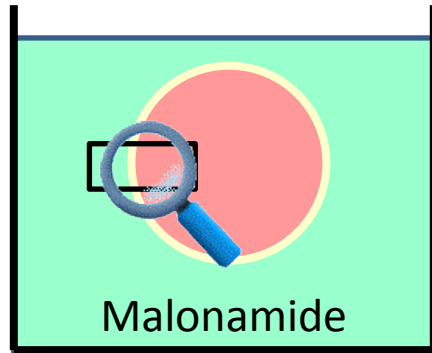


Question?

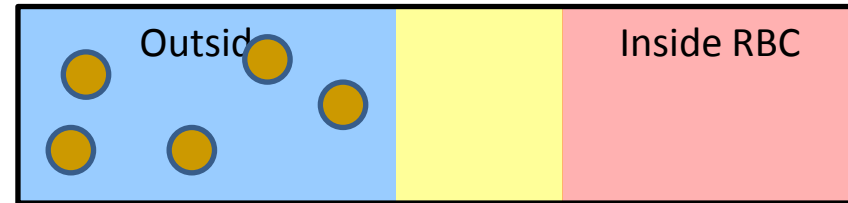
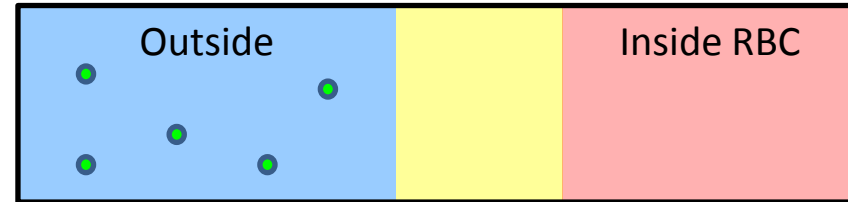
What is the primary factor that determines the diffusion rate of solutes through the Red Blood cell plasma membrane?

Is it A) the **size** of the solute particles
or is it B) their **solubility in lipids**?

Permeability of RBC membrane - Protocol



\neq lipid solubility
 \neq size



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

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

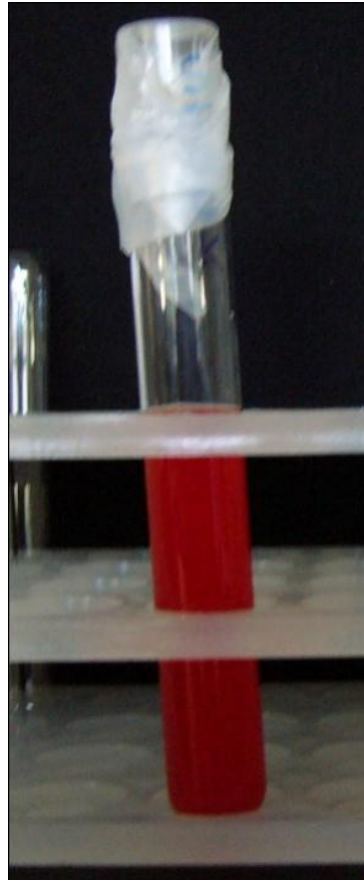
- **hemolysis time** is a good estimation of the diffusion rate
- **molecular size** (diameter) roughly parallels molecular weight.
- **the partition coefficient** of a solute is a measure of its relative solubility in water and lipids.

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

Permeability of RBC membrane - Data? Data analysis?

Permeability of RBC membrane - Data? Data analysis?

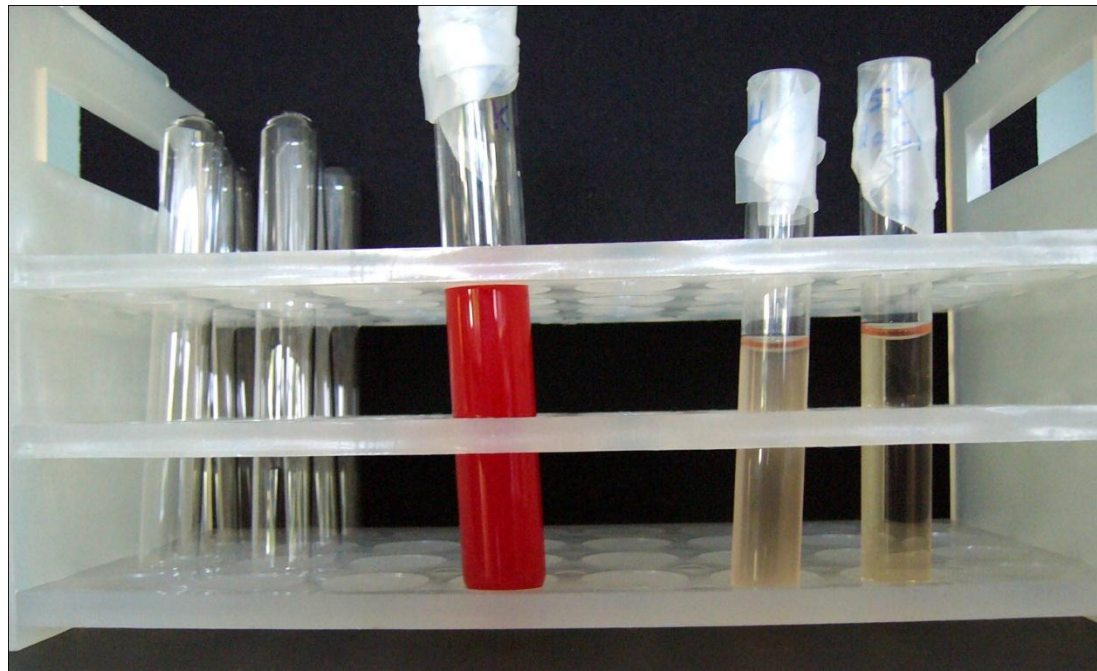
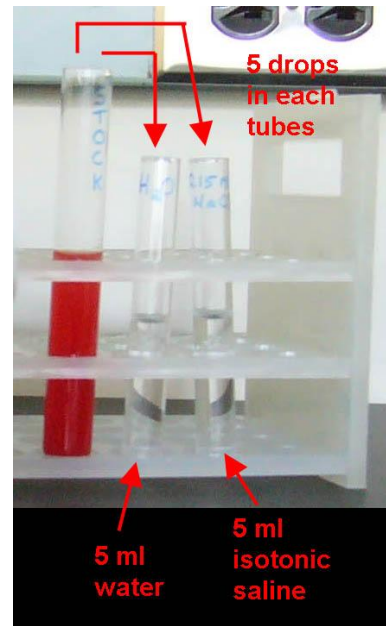
Permeability of RBC membrane - Methodology - Stock blood suspension



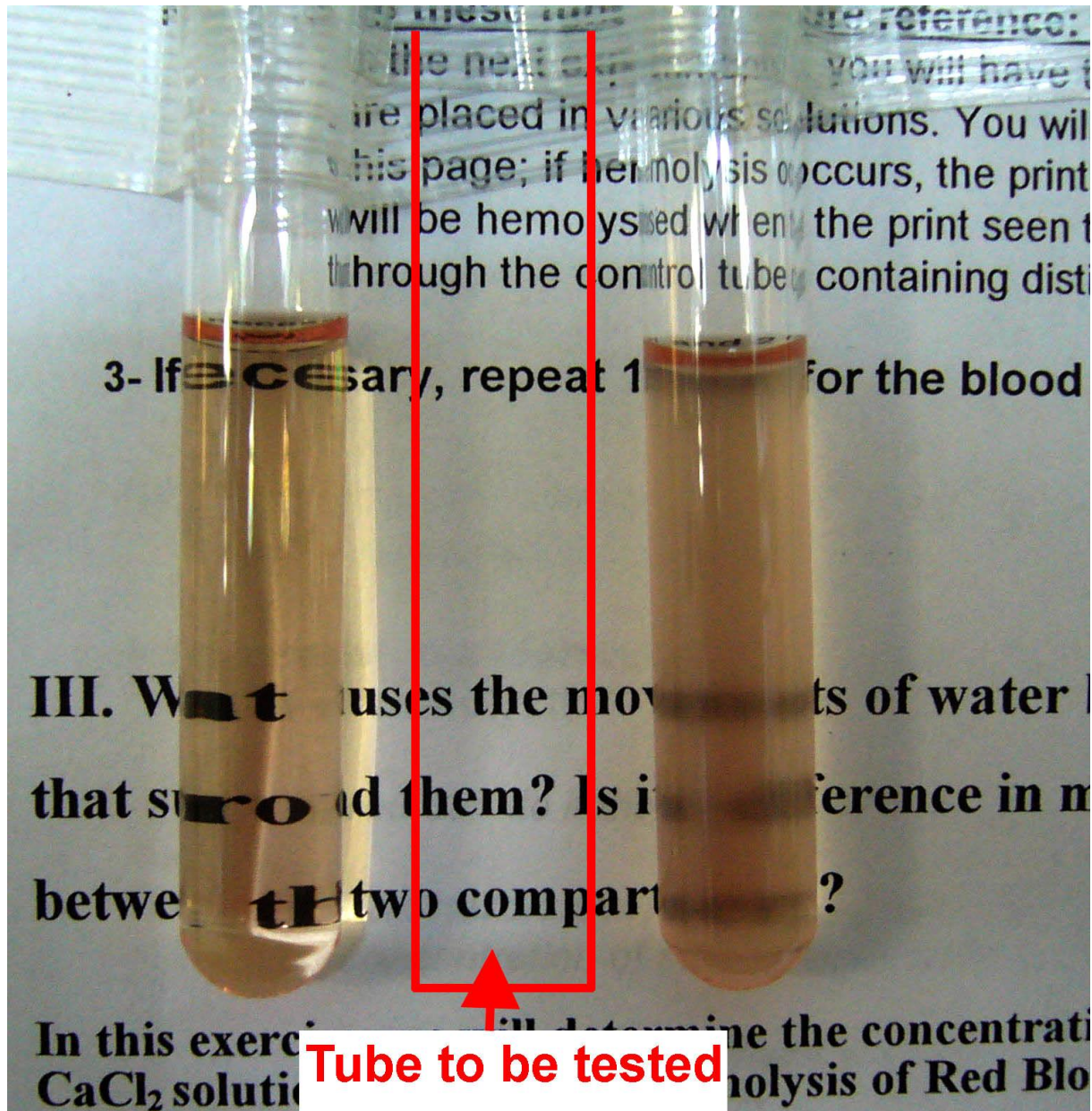
Steps to test for hemolysis

- 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



5 sheep
5 sets of solutions

Yellow:
missing
data

5 sheep

5 sets of solution

	A	B	C	D	E	F	G	H	I	J	K	L	M
3	OBJECTIVE 12												
4													
5	[Memorize time of the red blood cells in seconds]												
6	Station no	Groups	Glosser [duration]	glycerol	di-acetlin	tri-acetlin	malonamide	laetamide	dimethyl-urea	Succrose [duration]	Ethanol	Hydyl Alcohol	
7			glosser	glycerol	di-acetlin	tri-acetlin	malonamide	laetamide	dimethyl-urea	Succrose	Ethanol	Hydyl Alcohol	
8		HW	100	32	176	210	102	85	88	342.00	42	32	
9		PC	0.00030	0.00070	0.07100	0.40000	0.00000	0.00750	0.00200	0.00	0.00200	0.00370	
10	L11	1	550	330	5	3	240	104	10	550.00	5	2	Glosser and Succrose solutions did not hemolyse the blood cells
11	L11	2	380	270	5	10	240	120	5	800.00	5	2	Glosser and Succrose solutions did not hemolyse the blood cells
12	L11	3	750	265	15	11	200	154	15	440.00	10	8	Glosser and Succrose solutions did not hemolyse the blood cells
13	L11	4	370	265	27	23	307	120	3	570.00	10	12	Glosser and Succrose solutions did not hemolyse the blood cells
14	L11	5	550	276	10	12	200	174	13	560.00	3	7	Glosser and Succrose solutions did not hemolyse the blood cells
15	L11	6	660	303	14	13	311	130	10	660.00	10	13	Glosser and Succrose solutions did not hemolyse the blood cells
16	L11	7	660	467	3	5	350	150	3	600.00	5	4	Glosser and Succrose solutions did not hemolyse the blood cells
17	L11	8		253	30	10	230	141	11		5	3	Glosser and Succrose solutions did not hemolyse the blood cells
18	L11	9		201	16	14	262	116	8		20	23	Glosser and Succrose solutions did not hemolyse the blood cells
19	L11	10											
20													
21	L12	Groups	glosser	glycerol	di-acetlin	tri-acetlin	malonamide	laetamide	dimethyl-urea	Succrose	Ethanol	Hydyl Alcohol	COMMENTS
22	L12	1	infinitely	234	8	12	347	140	13	infinitely	11	10	Glosser and Succrose solutions did not hemolyse the blood cells
23	L12	2	infinitely	234	3	16	250	134	13	infinitely	8	6	Glosser and Succrose solutions did not hemolyse the blood cells
24	L12	3	infinitely	196	5	7	250	125	8	infinitely	5	10	Glosser and Succrose solutions did not hemolyse the blood cells
25	L12	4	infinitely	220	3	7	230	120	4	infinitely	17	10	Glosser and Succrose solutions did not hemolyse the blood cells
26	L12	5	infinitely	220	5	6	240	140	12	infinitely	15	10	Glosser and Succrose solutions did not hemolyse the blood cells
27	L12	6	infinitely	420	3	3	271	123	7	infinitely	7	7	Glosser and Succrose solutions did not hemolyse the blood cells
28	L12	7	560	330	7	10	235	150	10	560	12	3	Glosser and Succrose solutions did not hemolyse the blood cells
29	L12	8	515	267	1	1	302	127	1	535	12		Glosser and Succrose solutions did not hemolyse the blood cells
30	L12	9	720	340	15	7	200	124	8	600	3	6	Glosser and Succrose solutions did not hemolyse the blood cells
31	L12	10	330	233	10	14	270	153	13	1920	7	5	Glosser and Succrose solutions did not hemolyse the blood cells
32													
33													
34													
35													
36	L13	1	510	250	6	2	331	163	13	4710	13	5	Glosser and Succrose solutions did not hemolyse the blood cells
37	L13	2		265	15	3	200	214	21		8	10	Glosser and Succrose solutions did not hemolyse the blood cells
38	L13	3	4453	320	5	5	350	202	15	4453	8	7	Glosser and Succrose solutions did not hemolyse the blood cells
39	L13	4	4233	366	3	8	356	201	10	4233	3	24	Glosser and Succrose solutions did not hemolyse the blood cells
40	L13	5	4000	206	13	7	236	107	11	4000	10	5	Glosser and Succrose solutions did not hemolyse the blood cells
41	L13	6	4630	260	6	27	345	141	13	4630	10	13	Glosser and Succrose solutions did not hemolyse the blood cells
42	L13	7	4003	203	10	10	236	125	8	5121	7	5	Glosser and Succrose solutions did not hemolyse the blood cells
43													
44													
45													
46	L14	1	5100	297	36	24	256	143	36	4300	25	24	Glosser and Succrose solutions did not hemolyse the blood cells
47	L14	2	4000	225	10	10	224	163	6	4000	5	24	Glosser and Succrose solutions did not hemolyse the blood cells
48	L14	3	3923	250	8	16	245	173	8	3461	3	11	Glosser and Succrose solutions did not hemolyse the blood cells
49	L14	4	5200	216	5	4	217	130	10	5160	20	6	Glosser and Succrose solutions did not hemolyse the blood cells
50	L14	5	4020	235	1	6	225	132	6	4020	5	4	Glosser and Succrose solutions did not hemolyse the blood cells
51	L14	6											
52	L14	7	5455	216	1	10	257	167	15	5335	3	12	Glosser and Succrose solutions did not hemolyse the blood cells
53	L14	8	5040	245	1	3	203	143	36	3610	3	10	Glosser and Succrose solutions did not hemolyse the blood cells
54	L14	9	4543	240		6	277	165	10	4626	6	5	Glosser and Succrose solutions did not hemolyse the blood cells
55	L14	10	0026	222	12	16	221	136	13	5445	12	3	Glosser and Succrose solutions did not hemolyse the blood cells
56													
57													
58													
59	L15	1	5100	254	15	275	167	27	5100				Glosser and Succrose solutions did not hemolyse the blood cells
60	L15	2	1430	230	11	235	150	8	5730				Glosser and Succrose solutions did not hemolyse the blood cells
61	L15	3	5300	233	10	245	171	10					Glosser and Succrose solutions did not hemolyse the blood cells
62	L15	4	0204	373	24	405	177	40	6245	40	24		Glosser and Succrose solutions did not hemolyse the blood cells
63	L15	5	5501	210	21	297	144	20	2123	17	11		Glosser and Succrose solutions did not hemolyse the blood cells
64	L15	6											
65	L15	7	3360	174	12	197	115	13	3036	13	10		Glosser and Succrose solutions did not hemolyse the blood cells
66	L15	8	6734	210	12	10	253	141	10	6410	10	8	Glosser and Succrose solutions did not hemolyse the blood cells
67													

Permeability of RBC membrane - Data collected

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1																		
2																		
3		OBJECTIVE #2																
4																		
5				(Hemolysis time of the red blood cells in seconds)														
6	Section	Groups	Glucose (duration)	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl- urea	Sucrose (duration)	Ethanol	Methyl Alcohol						
7			glucose	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl-urea	Sucrose	Ethanol	Methyl Alcohol						
8		MW	180	92	176	218	102	89	88	342.00	42	32						
9		PC	0.000030	0.000070	0.071000	0.440000	0.000080	0.000750	0.002300	0.00003	0.032000	0.009700						
10	L11	1	6660	330	5	3	240	104	10	6600.00	5	2	Glucose and Sucrose solutions did not hemolyse the blood cells					
11	L11	2	900	270	5	10	240	120	5	840.00	5	7	Glucose and Sucrose solutions did not hemolyse the blood cells					
12	L11	3	7560	265	15	11	280	154	15	4440.00	10	8	Glucose and Sucrose solutions did not hemolyse the blood cells					
13	L11	4	5760	266	27	23	307	120	9	5760.00	10	12	Glucose and Sucrose solutions did not hemolyse the blood cells					
14	L11	5	5580	276	10	12	280	174	13	5640.00	9	7	Glucose and Sucrose solutions did not hemolyse the blood cells					
15	L11	6	6660	303	14	19	311	138	18	6660.00	10	13	Glucose and Sucrose solutions did not hemolyse the blood cells					
16	L11	7	6660	467	3	5	350	150	3	6480.00	5	4	Glucose and Sucrose solutions did not hemolyse the blood cells					
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19	L11	10																
20																		
21																		
22	L12	Groups	glucose	glycerol	di-acetin	tri-acetin	malonamide	lactamide	dimethyl-urea	Sucrose	Ethanol	Methyl Alcohols	COMMENTS					
23	L12	1	infinity	263	8	12	317	148	14	infinity	11	10	Glucose and Sucrose solutions did not hemolyse the blood cells					
24	L12	2	infinity	221	9	11	259	134	13	infinity	8	6	Glucose and Sucrose solutions did not hemolyse the blood cells					
25	L12	3	infinity	196	5	7	258	125	8	infinity	15	10	Glucose and Sucrose solutions did not hemolyse the blood cells					
26	L12	4	infinity	220	9	7	238	128	4	infinity	17	10	Glucose and Sucrose solutions did not hemolyse the blood cells					
27	L12	5	infinity	224	5	6	248	140	12	infinity	15	10	Glucose and Sucrose solutions did not hemolyse the blood cells					
28	L12	6	infinity	424	3	3	271	129	7	infinity	7	7	Glucose and Sucrose solutions did not hemolyse the blood cells					
29	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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