

**Cellular Biophysics**  
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**Blood Centrifugation**

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1. Sedimentation

2. Svedberg value, 70S Ribosome

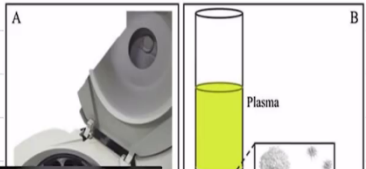
3. How to outrun diffusion

TODAY

Blood centrifugation- classical method

Pandey, Mehendale & Paul (2018)

Single-cell separation



The diagram shows a centrifuge on the left (labeled A) and a test tube on the right (labeled B). The test tube contains a yellow liquid labeled 'Plasma' and a small inset shows a microscopic view of cells.

Previously

1. Sedimentation

2. Svedberg value, 70S Ribosome

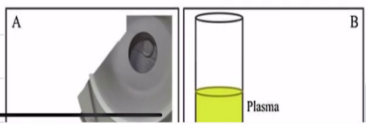
3. How to outrun diffusion

TODAY

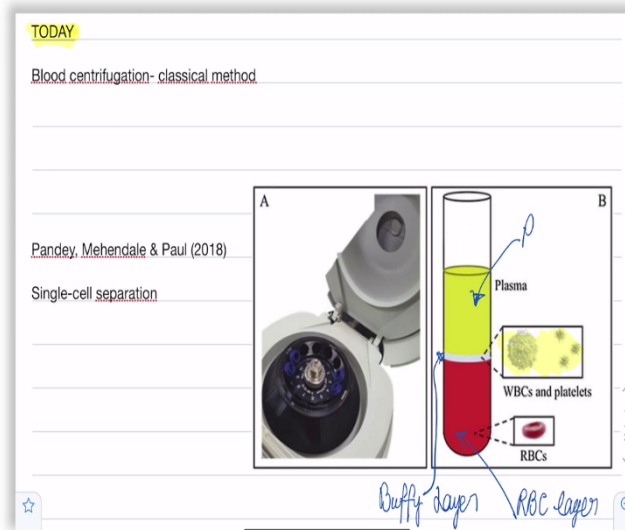
Blood centrifugation- classical method

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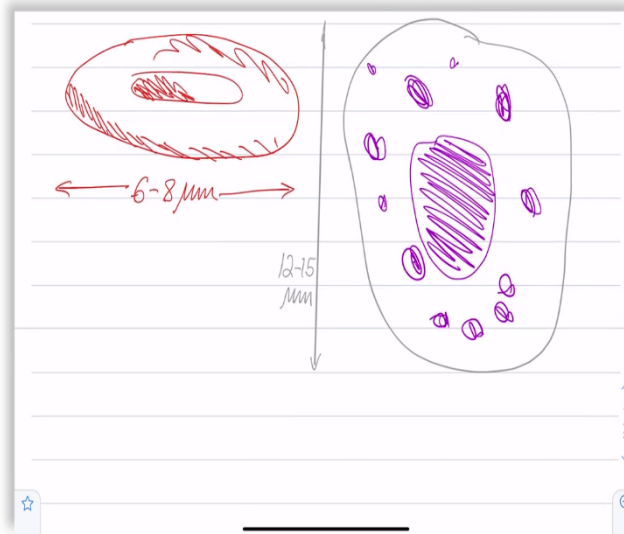
The diagram shows a centrifuge on the left (labeled A) and a test tube on the right (labeled B). The test tube contains a yellow liquid labeled 'Plasma' and a small inset shows a microscopic view of cells.



Hi, previously, we have discussed the topic of sedimentation, Svedberg's equation and value. Taking the example of the sedimentation velocity and centrifugation velocity of 70S ribosome and how in centrifugation, separation can outrun diffusion. For today, we are going to discuss blood centrifugation by the classical method and see how, now just laboratory centrifugation of the kind we would practice maybe in a basic biology lab, but also in a clinical setting, this might be relevant.

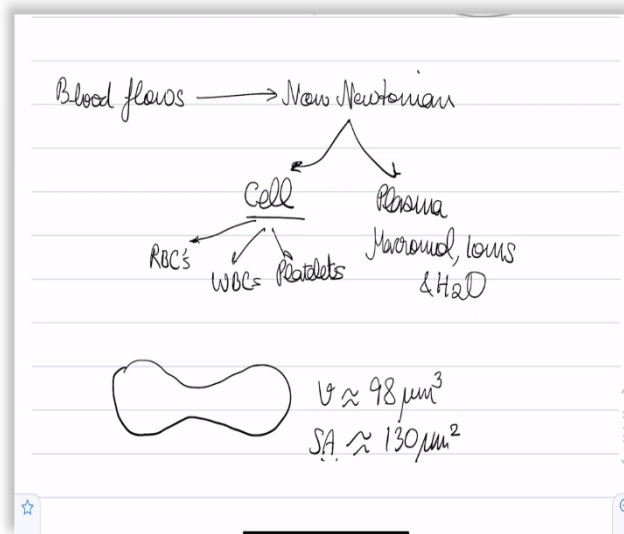
So, what you are looking at here is typical electric centrifuge, electronics centrifuge and the separation of citrated blood into plasma Buffy layer that is to say the white blood cells and platelets, this we can call Buffy layer and this is the RBC layer. This is because blood itself consists of three major centrifuge components or those that separate on centrifugation. The plasma which is sort of a clear fluid, the Buffy layer which contains the red blood cells and platelets and the red blood cells, which form a very large proportion.

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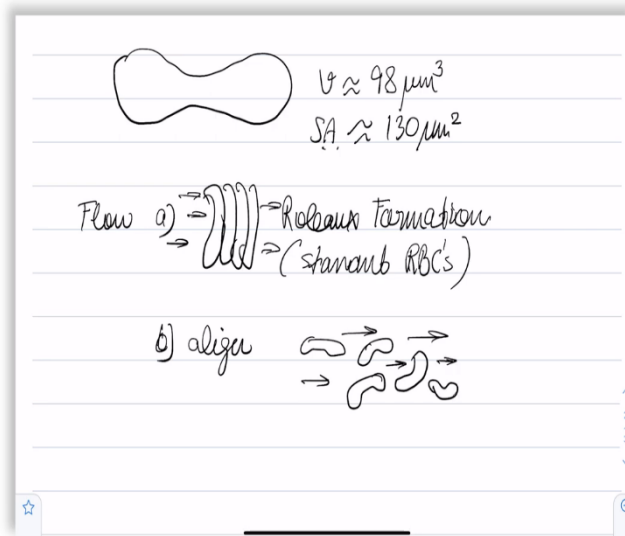
To remind ourselves red blood cells themselves are discoid structures which have a diameter of between 6 and 8 microns and indeed you will find that this size of the red blood cell is itself used as a metric for health. And in contrast, white blood cells can be large reaching 12 to 15 microns in them. They are also nucleated as opposed to RBCs that is mature RBCs in humans are denucleated they lack a nucleus. The granules I am drawing in the white blood cells are typically depending on the type of granule, neutrophils, basophils etcetera. They arise from the specific morphology of nucleophilic strain.

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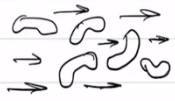
Now, blood itself is not static in terms of the fact that it flows and the flow is non-Newtonian. This is mostly because blood consists of these two primary components cellular components and plasma which is a mixture of macromolecules, ions and water. Cellular components are made of or consists of RBCs primarily the largest population, WBCs, platelets. The red blood cell size is also associated with a volume which is usually in the range of 28 micrometer cube and a surface area of 130 micrometer square.

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During this flow, you may observe that these discoid red blood cells form a sort of stack, stack of dishes. This is also referred to as Roleaux formation. It indicates stagnant RBCs resulting in this decade. The alternative is that they align in the direction of the flow, this is the flow direction when they are approximately aligned to the flow.

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b) align 

Human RBC concentration  $\xrightarrow{\text{avg.}}$  ♂  $4.5 - 5.5 \times 10^6$   
♀  $3.8 - 4.8 \times 10^6$

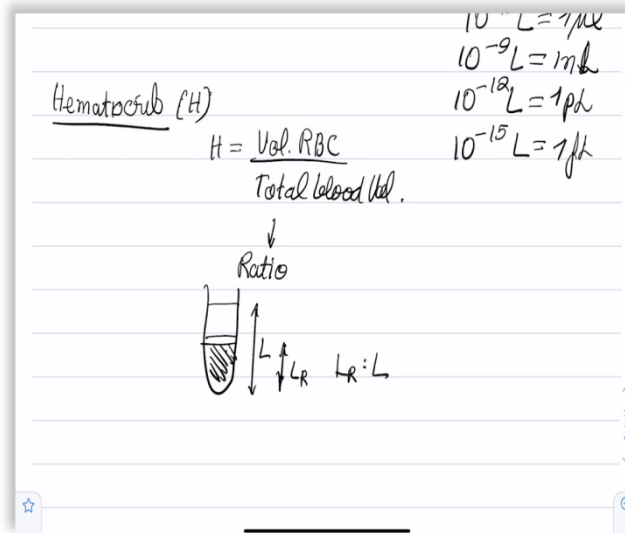
Mean Corpuscular volume (MCV)  $\frac{\text{Cells}}{\text{ml}} \rightarrow 10^{-6} \text{L}$

(MCV)  $\frac{\text{ml}}{\text{ml}} \rightarrow 10^{-6} \text{L}$

$\rightarrow 78 - 94 \text{ fL/RBC}$   $10^0 \text{ L}$   
 $10^{-3} \text{ L} = 1 \text{ ml}$   
 $10^{-6} \text{ L} = 1 \mu\text{l}$   
 $10^{-9} \text{ L} = 1 \text{ nl}$   
 $10^{-12} \text{ L} = 1 \text{ pl}$   
 $10^{-15} \text{ L} = 1 \text{ fl}$

Hematocrit (H)  $H = \frac{\text{Vol. RBC}}{\text{Total blood Vol.}}$

$\downarrow$   
Ratio



What is the typical content of RBCs in human blood concentration, we find that on an average the male population has between, 4.5 to 5.5 into  $10^6$  and female population on an average 3.8 to 4.8 into  $10^6$ . The unit is cells by micrometer. microliter is  $10^{-6}$  liters.

The mean corpuscular volume is a volume that is somewhat comparable to what we discussed here it is a 98 micrometer cube which translates into between 78 to 94 femtoliters per RBC. You will find it convenient to remember that the orders of magnitude go like this 10 to the 0 liter, 10 to the power minus 3 liters 1 ml, minus 6 liters is 1 micro liter, minus 9 liter is 1 nanometer, minus 12 for Pico liters, minus 15 for Femto liter. Hematocrit which is often something reported in a typical complete blood count is the volume of RBCs to the total blood volume.

Since this is a dimensionless number just a ratio. In theory, a centrifugation or even just sedimentation which allows the blood to settle down can be used to estimate hematocrit percentage by taking the ratio  $L_R$  to  $L$  from such a standardized tube. There are many references on clinical methods and physical and laboratory examination. And millet is one of the books that if you are interested in reading more, you can go back later part.

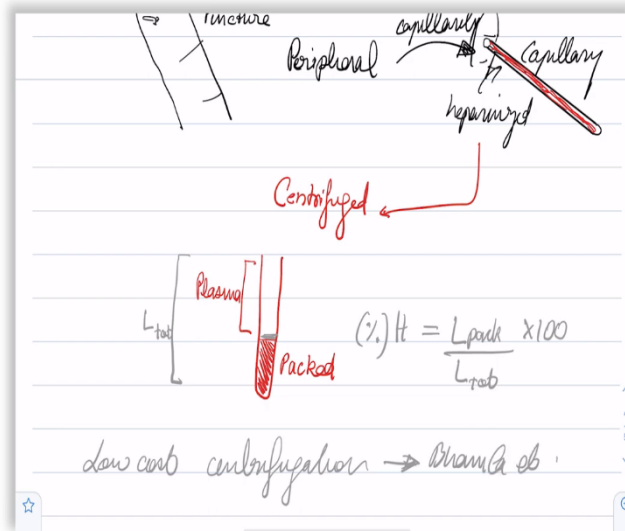
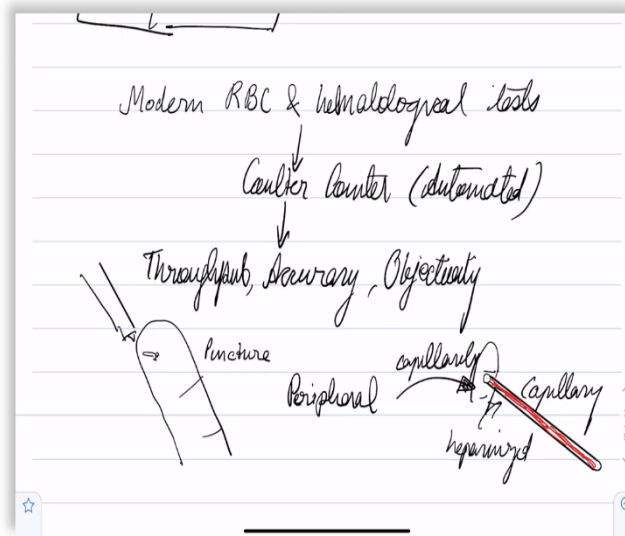
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		♀	♂
g/dl	[Hb]	12-16	14-18
	H(%)	36-48	40-54

Modern RBC & hematology tools  
 ↓  
 Coulter Counter (automated)  
 ↓  
 Throughput, Accuracy, Objectivity

In fact, as it turns out the hemoglobin concentration and the hematocrit, which can be reported also in percentage terms multiplied by 100 for females and male humans is reported to range for hemoglobin of 12 to 16 and 14 to 18. This is in grams per deciliter, whereas the hematocrit is 36 to 48 for females and 40 to 54 for males.

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Many of the modern RBC and hematological tests are done using Coulter counter that say automated which has many advantages in terms throughput, accuracy, objectivity in identification and few others. But for laboratory demonstrations, we still use conventional methods to try to understand the principle.

And the straightforward way that in the past when we have done experiments has involved taking a volunteer that is usually mean to subject themselves to auto phlebotomy a small pinprick has made, tiny amount of blood is released that droplet from the puncture wound in the peripheral blood vessel is taken by capillarity into a special tube that is made for these sort of experiments.



These are thin capillary tubes that are previously heparinized in order to prevent coagulation and by applying it to the wound the blood is sucked in, then this material is centrifuged and the packed volumes and plasma can then be absorbed into Buffy coat. this length will be L total, so, H then becomes L packed by L total into 100 to give you a percentage hematocrit. We will come back to this in the context of low cost centrifugation, in a paper that we will discuss by Bhamla everything al.