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ID: 2284123 SECTION: 2

**DIFFERENTIAL STAINING**

**Aim:**

The aim of this experiment is learning the blood cell. Also, learning how we distinguish between the type of white blood cell.

**Introduction:**

Staining material is organic and synthetic compound which is originated nitrobenzene or aniline. These materials which is dye can be acidic (anionic), basic (cationic), neutral. Generally, they made up of chromophore which gives color, auxochrome that is affect ionic character and color, and simple structural elements. There are different types of staining. Simple staining include just one type of dye and cationic dyes is used in this staining method. The other staining is negative staining and generally ionic dyes is used. The most important staining is differential staining in this experiment and this staining provide combining more then one dye. Also, all the dye which is used the differential staining stain different compartments and these areas stain different color.

Giemsa stain consists of combining different dyes. It includes three different dye which names are methylene blue, azure B, eosin Y. methylene blue is a cationic dye and it stain a compartment which is negatively charge. Azure B is used to stain azure eosin stain for the blood smear (Azure B, 2020). It works like metabolite of methylene blue. Lastly, eosin Y is an anionic dye and it stain basic compartment of the cell.

**Material/ Method:**

Collecting of blood sample:

* Slides are cleaned with %70 ethanol and let it out to dry.
* Slides are labelled at the end of the slides with the group number or table number or anything to remind you that you are.
* After drying, one drop of blood sample is put on towards end of the slide (close labelled end).
* Helping clean slide, clean slide with 35-45 degrees angle put on the slide with blood. Then, clean slide withdrawn until it touches the blood sample. After that, clean slide retracts the opposite direction until reach the other end of the slide quickly.
* The important thing is quick movement. If clean slide is not move quick, the image will not be clear. Some of the region would be layered and when this region is observed, visibility won’t enough.
* Let slides out to dry for a couple of minutes.

Fixation:

The purpose of the fixation is carried out the cell for permanently alive. Fixatives provide avoidance of autolysis and tissue putrefaction. If the fixative is not used, hydrolytic enzyme which is released by lysosome can be released and all the cell would be crumble. Fixation which is committed in the right way must provide all the cell part in soluble. Also, it provides avoiding swelling and shrinkage of the cell content. There are different type of fixative and one of them is methanol- filled fixative and it is used this experiment.

* Hellendahl jar is filled with methanol. Then, slides put into the jar.
* Methanol affect the pores so more pores will be formed. The result of this that sample stain easier.
* Hold on about 5 minutes.

Staining:

* After slides are removed from the Hellendahl jar, while the side of the slide is touched, get rid of the excess methanol.
* Hellendahl jar is filled with Giemsa stain which is 1/20 diluted with phosphate buffer (pH:6.8).
* It holds on about 30 minutes.

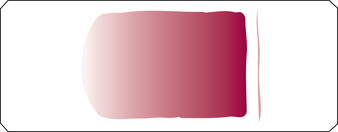
Washing:

* Get rid of the excess dye while the side of the slide is touched to filter paper or tissue paper.
* The slide wash with phosphate buffer which pH is 6.8.
* Last step in the washing is that, leave to dry in a vertical position.

Observation:

* Stained slides observe under 40X magnification.
* Take photo from different site of slide.
* Sample which observe the different region of sample can be change because sample is not the same density everywhere.

**Discussion:**



C- High density

1. Low density

B- Medium density

When we observe A site, the amount of WBC and RBC are less because as I pulled the slide and let it go, the last part that comes to the end is. Therefore, last part (A) have less blood cell which are WBC and RBC. We can observe the blood cell but not easy. Due to the low number of blood cell, we may be more likely to miss the cells. Also, when we look at the sample, we see lots of RBC. These cells do not have a nucleus and all the purpose providing transportation of oxygen. If these cells do not transport the oxygen, we cannot stay alive. Thanks to these cells, oxygen transport is occurred in our body. Because the oxygen is too necessary for our body, RBC’s are quite more. When we compare the WBC and RBC in terms of quantity, we realize that the amount of RBC is more than the number of WBC because of the importance of RBC as I told in the top of lines. The other part is B which have more density of blood cell when we compare to part A. Therefore, all blood cells are more. The density of blood cells is increased. We can see all the cell type clearly. The number of cells and proximity of cells are also increase. Again, if we compare RBC and WBC, the number of red blood cell is more than other blood cell. When we observe the C part, due to lots of blood cell, we cannot recognize the cells. We can think that, we observe clearer image because of the more blood cells. However, this idea is not true. Maybe, the blood cell may have coincided with one another. Therefore, we may not see lots of blood cell.

# When we observe the blood, which belonging to healthy person, we see that there are different proportions of the types of blood cells. When bone drop of blood is observed, we realize that there are 58.5% neutrophils, 3% eosinophils, 0.5% basophils, 34% lymphocytes and lastly 4% monocytes. The type of the white blood cell that a person examining blood under a microscope will see is the neutrophil (Extracting, Recognizing, and Counting White Blood Cells from Microscopic Images by Using Complex-valued Neural Networks,2012). Change of the number of these cells influence our body and it cause lots of health issue or opposite way, our health issue cause these changing. Here is the purpose of all the blood cells:

# Neutrophils serve as protection mechanism against the bacteria. These cells kill the bacteria. (Chen, 2020)

# Eosinophils kill parasites and serve allergic reactions. (Chen, 2020)

# Basophils have a role allergic reaction. (Chen, 2020)

# Monocytes throw out the bacteria cells with phagocytosis. Also, it serves throw out the dead, old, damaged cells in the body. (Chen, 2020)

# Lymphocytes are important for immune system. T lymphocytes have a role cell-mediated immunity. Also, B lymphocytes have roles at humoral immunity or anti-body production. (Chen, 2020)

# Therefore, when the allergic reaction is occurred in our body, the amounts of eosinophils and, basophils are increased (Basophil, Eosinophil & Mast Cell Disorders in Allergic Disease | World Allergy Organization, 2016).

# Giemsa interact with phosphate group of DNAs. Especially, the part which have more amount of adenine- thymine is attached and stained by Giemsa. Therefore, we understand that we observe the nucleus where the DNA is found so we can see the blood cells clearly. WBC have a nucleus and we observe the cells when we stain with Giemsa. The part which is found more adenine- thymine bond is becomes dark and other sites have light color. (Mokobi, 2020)

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