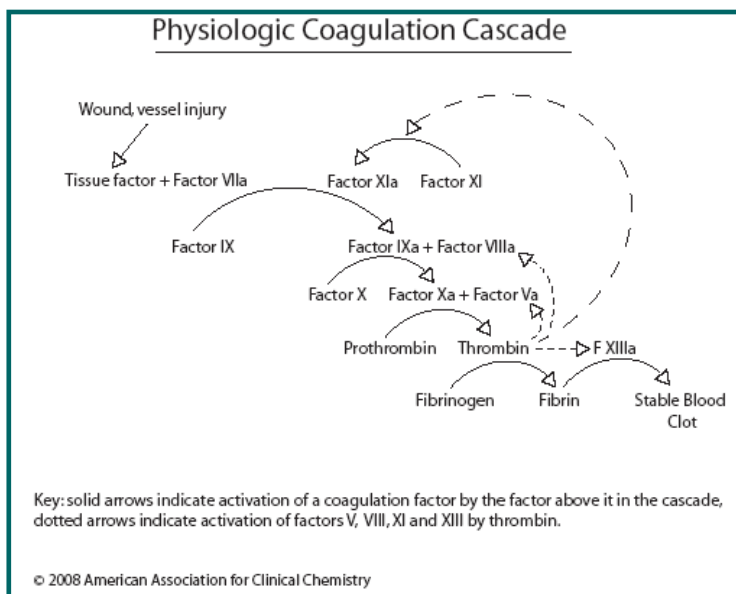




Fibrin – The Clotting Wonder

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Fibrin, also known as Factor 1a, is a protein involved in the clotting of blood. This fibrillar protein is polymerized to form a “mesh” which, in conjunction with platelets, creates a hemostatic plug or clot over a wound site. Fibrin is made from fibrinogen, a soluble plasma glycoprotein that is synthesized in the liver. During this process, called the coagulation cascade, zymogen (inactive enzyme precursor prothrombin) is activated and creates serine protease thrombin (pathway of activation). Fibrin, when crosslinked by Factor XIII (an essential clotting factor), forms a clot.



Fibrinogen also known as factor 1 is a 340kDa glycoprotein synthesized in the liver by hepatocytes and megakaryocytes.

◆ **Hepatocytes:** Make up 70-80% of the cytoplasmic mass of the liver. Hepatocytes are involved in protein synthesis, protein storage and transformation of carbohydrates, modification and excretion of external and internal substances. Hepatocytes display an eosinophilic cytoplasm, reflecting numerous mitochondria, and basophilic stippling due to large amounts of rough endoplasmic reticulum and free ribosomes. Brown lipofuscin granules are also observed (with increasing age) together with irregular unstained areas of cytoplasm; these correspond to cytoplasmic glycogen and lipid stores removed during

histological preparation. The average life span of the hepatocyte is 5 months; they are able to regenerate.

◆ **Megakaryocytes:** In general, megakaryocytes are 10 to 15 times larger than a typical red blood cell, averaging 50-100 μm in diameter. During its maturation, the megakaryocyte grows in size and replicates its DNA without cytokinesis (see next). As a result, the nucleus of the megakaryocyte can become very large and lobulated, which, under a light microscope, can give the false impression that there are several nuclei. Megakaryocytes are derived from hematopoietic stem cell precursor cells in the bone marrow. These stem cells live in the sinusoids of the marrow and are capable of producing all types of blood cells depending on the signals they receive. The primary signal for megakaryocyte production is thrombopoietin. Thrombopoietin is necessary for inducing differentiation of cells in the bone marrow towards a final megakaryocyte cell type. The cell eventually reaches megakaryoblast stage and loses its ability to divide. Once the cells have completed differentiation and become mature megakaryocytes, they begin the process of producing platelets. Thrombopoietin plays a role in inducing the megakaryocyte to form small proto-platelet processes. Platelets are held within these internal membranes within the cytoplasm of megakaryocytes. In either scenario, each of these proto-platelet processes can give rise to 2000-5000 new platelets upon breakup. Overall, 2/3 of these newly-produced platelets will remain in circulation while 1/3 will be sequestered by the spleen. After budding off platelets, what remains is mainly the cell nucleus. This crosses the bone marrow barrier to the blood and is consumed in the lung by alveolar macrophages.

◆ **Cytokinesis:** Is the process when the cytoplasm of a single eukaryotic (membrane bound nucleus) cell is divided to spawn two daughter cells. It usually starts during the late stages of mitosis, and sometimes meiosis, splitting a binucleate cell in two, to ensure that chromosome number is maintained from one generation to the next.

What does this mean for fibrin's role in disease? Overproduction of fibrin due to the activation of the coagulation cascade leads to thrombosis, while decreased or underproduction of fibrin predisposes us to hemorrhage.

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Abnormal fibrin levels or clotting processes are detected and monitored with simple routine venous blood samples. Disease or dysfunction of the liver, as well as abnormal fibrin levels, may and can lead to either one of the following:

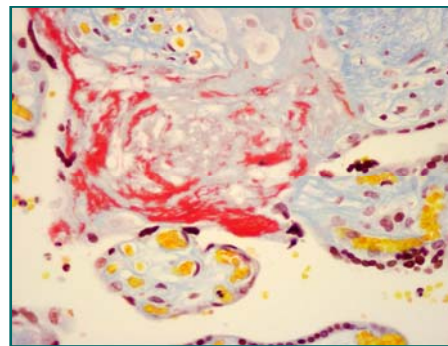
- ◆ **Thrombosis:** Is the formation of a clot or thrombus within the lumen of a blood vessel, obstructing the flow of blood through the circulatory system. When a thrombus occupies more than 75% of the lumen of an artery, blood flow to the tissue supply is reduced enough to cause symptoms because of decreased oxygen (hypoxia) and accumulation of metabolic products like lactic acid. More than 90% of obstruction can result in anoxia, the complete deprivation of oxygen, and infarction, a mode of cell death.
- ◆ **Hemorrhage:** Also known as bleeding or the loss of blood from the circulatory system. Bleeding can occur internally, where blood leaks from blood vessels inside the body or externally, either through a natural opening, or through a break in the skin. Increased production of fibrin can effect or create disease processes in the cardiac, renal and musculo-skeletal (arthritis) systems while the decreased production of fibrin can be related to placental abruption, hypoxia, or liver disease (cirrhosis). Histologically, lesions left from fibrotic activity can be detected utilizing various special stains including the Martius Scarlet Blue (Lendrum et al, 1962) stain for connective tissue and fibrin.

The Martius Scarlet Blue (MSB) stain contains four dye components:

- ◆ **Martius Yellow:** Acid yellow 24, Manchester yellow, or C.I. 10315 is a dye with the molecular formula $(O_2N)_2C_{10}H_5OH$. It is soluble in water and slightly soluble in ethanol. Is used to stain erythrocytes in connective tissue stains to provide higher contrast. It usually comes as disodium salt. NFPA: Health = 2 / Flammability = 0 / Reactivity = 0.
- ◆ **Ponceau 6R:** Crystal Ponceau 6R, Crystal scarlet, Brilliant crystal scarlet 6R, Acid red 44, or C.I. 16250 is a red azo dye with the molecular formula $C_{20}H_{12}N_2Na_2O_7S_2$. It is soluble in water and slightly soluble in ethanol. It is used as a food dye, with E number E126. It usually comes as disodium salt. NFPA: Health = 0 / Flammability = 0 / Reactivity = 0.
- ◆ **Methyl Blue:** Cotton Blue, Helvetia blue, Acid blue 93, or C.I. 42780 is a dye with the molecular formula $C_{37}H_{27}N_3Na_2O_9S_3$. Methyl blue stains collagen blue in tissue sections. It is soluble in water and slightly soluble in ethanol. It is used in differential staining. Methyl blue *should not be confused* with methyl violet or methylene blue, two completely different dyes. NFPA: Health = 1 / Flammability = 0 / Reactivity = 0.

- ◆ **Celestine Blue:** Mordant Blue 14, C.I. 51050 is a dye with the molecular formula of $C_{17}H_{18}ClN_3O_4$. The ferric mordanted celestine blue B attaches to DNA phosphate groups. This ferric mordanted nuclear stain is resistant to decolorization by sequential acid stains and solutions. NFPA: Health = 1 / Flammability = 0 / Reactivity = 0.

The principle of this stain is that Martius Yellow, a small molecule dye, together with phosphotungstic acid in alcoholic solution stains red cells. Early fibrin deposits may be colored, but the phosphotungstic acid blocks the staining of muscle, collagen, and connective tissue fibers. Crystal Ponceau 6R, a medium sized molecule stains muscle and mature fibrin. Phosphotungstic acid removes any red stain from the collagen. The large molecule dye Methyl Blue stains the collagen and old fibrin. This stain can be performed on formalin fixed paraffin embedded tissue, and if needed can be enhanced by mordanting in Bouin's Fluid for 1 hour at 60° Celsius prior to the staining protocol. If the connective tissue appears to be overstained with the



MSB Stain for fibrin

Ponceau 6R, increase the 1% phosphotungstic acid incubation and check repeatedly under the microscope for clearance of the connective tissue prior to continuing on with the stain. Our laboratory stumbled across this on the web searching for a

good stain to demonstrate fibrin. We have found it to be simple and reproducible with excellent results; sometimes even an oldie can be a goodie!



REFERENCES:

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MARTIUS SCARLET BLUE STAIN

REAGENTS

WORKING CELESTINE BLUE SOLUTION:

Dissolve 5.0 grams ferric ammonium sulphate (iron alum) in 100.0 ml distilled water without the aid of heat. Add 0.5 grams Celestine B and boil for 3 minutes. Cool, filter and add 14.0 ml glycerin. This reagent is stable for 6 months.

STOCK SOLUTION A (HEMATOXYLIN)

Dissolve 1.0 gram hematoxylin (CI 75290) in 5.0 ml distilled water. Add 95.0 ml of 95% ethanol. Store in brown bottle. Stable 6-12 months.

29% FERRIC CHLORIDE

Dissolve 29 grams of ferric chloride in 100.0 ml distilled water. Store at room temperature. Stable 1 year.

STOCK SOLUTION B (FERRIC CHLORIDE)

Mix 4.0 ml 29% ferric chloride with 95.0 ml distilled water. Slowly add 1.0 ml concentrated hydrochloric acid (HCl). Store at room temperature. Stable for 1 year.

WORKING WEIGERTS HEMATOXYLIN:

Stock Solution A (Hematoxylin) - 25.0 ml
Stock Solution B (Ferric Chloride) -25.0 ml
Mix and Filter prior to use.

1% ACID ALCOHOL

Slowly add 1.0 ml concentrated HCl to 99.0 ml 70% reagent or ethyl alcohol. Mix together. Stable 3-6 months.

WORKING MARTIUS YELLOW SOLUTION:

Dissolve 2.0 grams of phosphotungstic acid in 100.0 ml 95% ethanol followed by the addition of 0.2 grams Martius Yellow, mix until dissolved. Store at room temperature. Stable for up to 1 year.

WORKING CRYSTAL PONCEAU 6R SOLUTION:

Dissolve 1.0 gram of Ponceau 6R in 100.0 ml 2% aqueous glacial acetic acid. Store at room temperature. Stable for up to 1 year.

WORKING 1% AQUEOUS PHOSPHOTUNGSTIC ACID: (MAKE FRESH EACH USE)

Dissolve 1.0 gram of phosphotungstic acid in 100.0 ml distilled water.

WORKING METHYL BLUE SOLUTION:

Dissolve 0.5 grams Methyl blue in 100.0 ml 1% aqueous glacial acetic acid. Store at room temperature. Stable for up to 1 year.

STAINING PROTOCOL

1. Deparaffinize and hydrate to distilled water.
2. Working Celestine Blue solution - 5 minutes.
3. Wash in running tap water 5 minutes.
4. Freshly filtered Weigerts Hematoxylin - 5 minutes.
5. Rinse in running tap water for 5 minutes.
6. Differentiate in 1% Acid Alcohol – 10 to 20 seconds.
7. Rinse and blue in running tap water for 5 minutes.
8. Rinse in 95% Ethanol several changes.
9. Blot excess alcohol on absorbent toweling. (Don't let dry)
10. Working Martius Yellow solution – 2 minutes.
11. Rinse slides in tap water several changes.
12. Rinse in distilled water.
13. Working Crystal Ponceau 6R solution – 10 minutes.
14. Rinse in distilled water.
15. Differentiate in 1% Phosphotungstic Acid – 5 to 10 minutes, or until the red dye comes out of the connective tissue.
16. Wash in distilled water – 30 seconds.
17. Methyl Blue solution – 2 to 5 minutes.
18. Rinse in distilled water.
19. Dehydrate, clear and mount.

RESULTS

Fibrin – Red (Early fibrin may color yellow and very old fibrin may stain blue)
Muscle Fibers – Deeper red than fibrin
Nuclei – brown/black
Red blood cells – yellow
Collagen – blue



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DATE OF ARTICLE: Fall 2008
TITLE: Fibrin: The Clotting Wonder!!
AUTHOR: Amy S. Porter, HT(ASCP), QIHC

DIRECTIONS:

1. Answer the following questions by circling the one (1) BEST answer for each question.
2. Complete the information required at the bottom of the page.
3. Submit questions & check made out to "**MSH**"(in US funds) to: Peggy Wenk, 3840 Elmhurst Rd., Waterford, MI 48328
4. To earn CE from MSH, completed form must be submitted within **3 years (36 months) of original date of article.**

1. Fibrinogen is made in which of the following?
 - A. Alveolar macrophages
 - B. Hepatocytes
 - C. Lymphocytes
 - D. Paneth cells
2. Overproduction of fibrin can cause:
 - A. Cirrhosis
 - B. Hemorrhage
 - C. Hemosiderosis
 - D. Thrombus
3. With the MSB stain, red blood cells are stained with which of the following?
 - A. Celestine blue
 - B. Martius yellow
 - C. Methyl blue
 - D. Ponceau 6R
4. TRUE or FALSE (circle one): When stained with the MSB procedure, fibrin can end up being stained different colors, depending upon the age of the fibrin.

PLEASE PRINT NEATLY

Date Submitted (mm/dd/yy): _____

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