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Microanatomical Stains and procedures: Review

Abdulrazzaq baqer kadhim[1] Eman fasial Al-Baghdady[2] Eman Ibrahim Dli[3]
Anatomy and histology Department College of Veterinary Medicine, University of Al-Qadisiyah, Iraq
abdulrazzaq.alrabei@qu.edu.iq
07812352090

Abstract

The purpose of staining is to enhance the visibility of parts of cells or tissues. Consequently, while analyzing uncoated static models or slices, the distinguishable components have different refractive indices. Similar refractive indices render parts indistinguishable. But after dying it with one or more dyes, the color of the dye will change and increase the distinction between the parts of different refractions; similarly, dyes that color parts of the same refraction with similar refractions with different colors or varying degrees of the color of the same dye will make the differences between the chemical or physical components of those parts more apparent, thereby making them appear more distinct. The purpose of the study was to shed light on how histological and histochemical stains are currently being treated.

Keyword: staining, PAS, Tissue processing

Introduction

The histological study of animal and plant cells and tissues under a microscope by staining and slicing them (electron or light magnifying lens) is called histology. Two hypotheses were given to explain the tissue staining rules.

1-The chemical theory announced by Ehrlich in 1881 and stating that the formation of tissue is based on the formation of salt between the positive cation or the negative anion dye and specific chemical groups in the cell or tissue. They are either acidophilic and include basic groups, in which case they interact with acidic dyes, or they are either basophilic and contain acidic groups, in which case they interact with base pigments.

2-Absorption, desorption, capillary associations, capillary action, and osmotic diffusion are examples of natural theories. The current sentiment is related to chemical and natural coloring techniques.

Factors affecting staining

1- Temperature
2- mordants
3- Prepare the stain and storage
4- The intensity of the pigment
5 - the rate of ionization for each commander.
6- The pH of both the dye solution and protein tissues
7 - The type of stabilizer used
8 - The alcoholic or aqueous nature of the dye solution
9- If the dye is single, then it is single, double, or multiple, then it is multiple.
10- The dye concentration in the solution was low or high (1,2,3)

Staining is a technique used to heighten the contrast of a substance, often on a microscopic size. Histology. Histopathology, hematology, and cytopathology are medical specialties that focus on microscopy and disease diagnosis. They all regularly make use of stains and dyes in their work. Stains can be used to distinguish between different types of biological tissues (such as muscle fibers or connective tissue), different types of cells (such as blood cells), and different organelles within a single cell. The addition of a dye to a substrate is used in biochemistry to determine the presence or concentration of a specific component, such as DNA, proteins, lipids, or carbohydrates. Similar effects can be achieved using fluorescence staining and labeling. Flow cytometry and gel

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electrophoresis also use biological staining to mark proteins or nucleic acids. (3,4). Using either bright-field or fluorescence illumination, light microscopes are utilized for high-magnification studies of stained samples. Because staining can also be used to analyze the structure of non-biological substances, such as the lamellar structures of semi-crystalline polymers or the domain structures of block copolymers, it is not limited to the study of biological substances alone. (5) Staining is caused by largely salty organic mixtures with numerous physiognomies. All stains fall into two major categories: A color-preferred atomic group known as chromophores and a group that may chemically combine dyes with compounds and is known as color mask auxochromes. A: Stains can also be classified based on pH

1- Basic stains when the dye has an organic base. Combining with non-colored tissue acidic radicals, such as acetate radicals, chlorides, or sulfites. These dyes are either water-soluble, alcohol-soluble, or both. Examples include and. staining with hematoxylin and (Safranin O).

2- Acid stains are colors with acidic origins. A colorful organic acid that interacts with non-tissue-coloring base metals to generate a compound. Typically sodium or potassium. The pale green dye yellowish, green light, and eosin stains are examples of stains that dissolve in either water, alcohol, or both.

3- Neutral stains consist of both acidic and basic stains. In which both positive and negative ions form groups of the chromophores of the color carrier. Typically, these colors dissolve in alcohols, but they also dissolve in water. They are colloidal solutions, neutral red being an example.

4- The characteristics of color carriers Nitrous stains such as naphthol green B, Azo stains such as Orang G, and Aryl methane are comparable to rapid green, a class of acridine dyes that includes acridine orange stains as an example. A class of imine-quinine dyes, of which safranin O is an example

5- The classification of pigments according to the propensity of protoplasmic components to stain. A. Nuclear pigments are pigments with a propensity to stain the nucleus. Due to their high nucleic acid content, they tend to stain with basal pigments and Basic spots. The region is indicated initially. The disposition to stain with basophilic pigments is known as basophilic. They were diverse parts of various body parts. The colored insoluble nuclear pigments of savory salts are fundamental dyes. B. Cytoplasmic staining consists of pigments of a basal origin that gravitate to the cytoplasm. To dyeing with acid dyes. All sections are characterized as cytoplasmic parts basal Acidophilus. When treated with acid dyes, these parts retain their base hues and are not melted (5,6,7)

Then proceed with the routine histological technique by way of follows:

1) Washing: Two hours were spent in tap water.

2) Dehydration: Two hours were spent in each concentration putting the specimens through a graded sequence of ethanol (70% - 80% - 90% - 100%) to eliminate the water.

3) The clearing is accomplished by passing the specimens through xylene twice for (1-30) minutes at each step.

4) Infiltration: In two steps, the specimens were transferred to molten paraffin wax. Each step rests in the oven at 54-64°C for two hours.

5) Embedded: The specimens were embedded in paraffin wax using plastic cast molds in preparation for sectioning.

5) Sectioning: The specimens were sectioned using a rotary microtome with a section thickness of 5-6 m. The histology sections were placed in a water bath heated to 52°C

6) Mounting: The slices were removed from the bath and placed on slides coated with Mayer's albumin Mixture of egg albumin and glycerin with a 1:1 ratio. A small amount of thymol was added. The slides were then baked at 40 °C for 24 hours (Luna, 1968).

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Histological sections were stained using the following stains:

1- Harris Hematoxylin and Eosin stain Hematoxylin and Eosin stain

For general Histological structure

To exhibit collagen and smooth muscle fibers by Massons Trichome stain

3-Verhoffs stain: To exhibit elastic fibers

4-PAS To detect mucopolysaccharides, glycoproteins, and basement membranes

5-Alcian Blue (pH 2.5): To detect mucopolysaccharides and glycoproteins. (8,11) Hematoxylin is one of the most frequent blood vessel stains. In South and Central America, a little leguminous tree called Hematoxylin is farmed for its wood, from which microscopic preparations are produced. The dye is removed from the stems by applying a b After drying and dissolving ether in water, it is filtered and crystallized. And as these are measures Hematoxylin is among the most expensive dyes since it is long and costly. Hematoxylin is never applied As a dye because of its affinity for tissues, unless oxidized to hematein. Consequently, of these Hematoxylin loses two hydrogen atoms during oxidation. Normal alcoholic or aqueous oxidation of hematoxylin takes three to four months; this process is known as aging quickly by adding oxidizing agents such as Ripening. This oxidation can be accomplished industrially using hydrogen peroxide, mercury oxide, potassium iodate, and potassium permanganate (11). Masson's trichrome. The numerous formulations that were produced based on the original formulation that was developed by Claude L. Pierre Masson (1880-1959) have a range of dissimilar applications, but they may all be used to differentiate cells from the connective tissue that surrounds them. The majority of recipes produce muscles fibers that are rosy, the collagen and bones that are azure or green, cytoplasm that is pink, light red, and nuclei of cells that are brown to black.In order to apply the trichrome, first the fixed specimen is submerged in Wiegert's iron hematoxylin. Next, the following solutions are added to the specimen: The three solutions that makeup Wiegert's hematoxylin are as follows: ferric chloride in watery hydrochloric acid hematoxylin trendy ethanol with a concentration of 95%; and potassium ferric solution alkalinized with sodium borate. It is utilized in the process of nuclear staining. Acid fuchsine, Xyloidines Ponceau, acetic acid, and water are the components of Solution A, which is more often referred to as plasma stain. Other red acid dyes, such as Biebrich scarlet, may also be utilized in the production of Lillie's trichrome. Solution b is composed of phosphomolybdic acid that has been dissolved in sterile water. When it comes to staining collagen, Solution c, also known as fiber stain, will either have Light Green SF or Fast Green FCF in its composition. Methyl blue or water blue are two alternatives that might be used in its place if blue rather than green is the desired color. (27). Verhoeff stain is a method of staining that is utilized in the field of histology. It was invented in the year 1908 by Frederick Verhoeff. (1874–1968). Since elastic has a robust attraction to iron-hematoxylin mix that is generated by the stain's ingredients, it will keep the dye for a longer period of time compared to the other components of the tissue. Because of this, elastin is able to maintain its color even after the decolorization of the other components of the tissue. Collagen fibers are dyed red, elastic fibers are stained black, cell nuclei are discolored yellow, and the cytoplasm and other tissue elements are discolored yellow. Sodium thiosulfate is utilized in the process of getting rid of any excess iodine, and Van Gieson's stain is utilized in the process of providing contrast to the primary stain. (25).Periodic acid–Schiff : Enables the discovery of polysaccharides, like glycogen, mucosubstances, glycoproteins, glycolipids, and mucins are all included in this category. Periodic acid oxidizes the nearby diols present in sugars, there by separated the

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interconnected between two elements carbon that is not complicated in the glycosidic connection or ring end in the circle of the monosaccharide components that are part of the extended polysaccharides and founding a couple of aldehydes at the two allowed tips of each wrecked monosaccharide ring. This results in creation of a long polysaccharide chain In order to avert any extra oxidation of the aldehydes, it is essential that the oxidation condition be effectively managed. These aldehydes, when combined with the Schiff reagent, produce a color between magenta and purple. In order to create a counterstain, an appropriate base stain is normally applied first. The PAS is a combination of the PAS stain with the enzyme diastase, which breaks down glycogen (14).Alcian blue Alcian blue is recognized as Etch blue 1 and C.I. 74240, originally identified as Alcian blue has factually been the most main and trustworthy member of a family of polyvalent basic colors. Stains acidic polysaccharides like glycosaminoglycan. It is a popular cationic color for light and electron microscopes for several of these targets. Histology has traditionally stained paraffin-embedded and semi-thin resin slices with alcian blue for light microscopy. "Alcianophilic" tissue stains blue to bluish-green. Alcian blue, H&E, and PAS, staining can be combined. Alcian blue can stain glycoproteins on polyacrylamide gels or western blots and quantify acidic glycan in solution using microspectrophotometry (19). Sliver: The staining of histologic sections with silver is known as silver staining. This type of staining is necessary for displaying proteins and DNA. It is used to demonstrate equally intracellular and extracellular molecules. Silver stain is also practical in temperature-grade electrophoresis. Following formalin fixation, argentaffin cells adapt silver solution to silver metal. Golgi's technique was created by Camillo Golgi, who practical a response the silver nitrate to potassium dichromate for precipitate silver chromate in approximately cell. When argyrophilic cells are exposed to a stain containing a reductant, the silver solution is converted into metallic silver. Consider hydroquinone and formalin as examples. (17). Sudan3 Sudan3 Staining occurs when Sudanese dyes are used to stain Sudanese materials, typically fats. Sudanese 3 and Sudanese 4 Oil Red O are commonly utilized. Sudan staining is commonly utilized to quantify adipose tissue in basment membrane of cells (29).Safranine (or safranine O)Looks like it's blood-red. It binds nuclei (DNA) and a extensive diversity of polyamines originate in tissues, such as the glycosaminoglycans . In cartilage and mast cells, and pledges lignin and plasticity mechanisms in plants. (21) In contrast to other dyes that impart blue and red hues to the nuclei and cytoplasm of animal (including human) tissues, safranine imparts a distinctive yellow color to collagen (30)5. Results and Discussion. Histopathology has advanced significantly, according to a review of the literature on staining techniques. Hematoxylin and other specific stains were frequently used as staining agents (3). Although these staining techniques are still in use today, their efficacy has been enhanced through a number of modifications. Due to its toxicity, certain previously known stain removal techniques were abandoned. Several ways for staining have been developed to advance staining methodology. There is an increasing demand for efficient, accurate, and less burdensome staining methods (21). The modern histopathology laboratory is overburdened with a wide variety of histological activities (17). Therefore, in order to make accurate conclusions, the greatest histologists have a superior awareness of diverse stains for certain applications (22). The enlarged mechanical development of a magnifying lens, the creation of the histologic stains plant in Germany in 1856, which generated a range of new-histological stains, resulting in an enormous

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change and improvement in histologic stains throughout the entirety of the history of histology (18). These pathologists created intraoperative staining procedures for frozen tissue segments by modifying a histopathology-specific staining approach (23). The paraffin penetration staining technique was invented at this period (15). Despite these advancements, some old staining techniques are still in use, though others have been substituted via immunological or staining innovations. In addition, the difficulty of stains has augmented in order to simplify rapid and predictable staining procedures that divulge fine and separate tissues (24).

5. Results and Discussion

Histopathology has advanced significantly, according to a review of the literature on staining techniques. Hematoxylin and other specific stains were frequently used as staining agents (3). Although these staining techniques are still in use today, their efficacy has been enhanced through a number of modifications. Due to its toxicity, certain previously known stain removal techniques were abandoned. Several ways for staining have been developed to advance staining methodology. There is an increasing demand for efficient, accurate, and less burdensome staining methods (21). The modern histopathology laboratory is overburdened with a wide variety of histological activities (17). Therefore, in order to make accurate conclusions, the greatest histologists have a superior awareness of diverse stains for certain applications (22). The enlarged mechanical development of a magnifying lens, the creation of the histologic stains plant in Germany in 1856, which generated a range of new-histological stains, resulting in an enormous change and improvement in histologic stains throughout the entirety of the history of histology (18). These pathologists created intraoperative staining procedures for frozen tissue segments by modifying a histopathology-specific staining approach (23). The paraffin penetration staining technique was invented at this period (15). Despite these advancements, some old staining techniques are still in use, though others have been substituted via immunological or staining innovations. In addition, the difficulty of stains has augmented in order to simplify rapid and predictable staining procedures that divulge fine and separate tissues (24).

6. Conclusion:

Histological staining is common in scientific and obsessive studies. Histological staining involves fixation, handling, insertion, segmentation, and staining. Histologists used readily available synthetics such as potassium dichromate, alcohol, and mercuric chloride for microscopic examination. After sharp fixatives and staining agents, dazzling staining agents were invented. Stains included carmine, alcian blue, silver, trichrome, PAS, and hematoxylin. Due to synthetic, atomic scientific testing and immunological systems like Histochemistry, histological staining has advanced, making organ and tissue analysis easier. Hematoxylin, a common stain color used in histology, stains cell cores bright blue, while eosin stains them pink. Despite these advances, certain historical staining procedures are still utilized, while others have been replaced by immunostaining or staining. Because synthetic chemicals were dangerous for clinical research, some staining methods were discontinued. Changes in responsibilities necessitated new staining methods. Modern histology uses a variety of staining methods to improve staining, according to contextual studies. Modern histology stains have been replaced or combined to improve their performance.

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