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A HISTORY OF EVOLUTION OF SPECIAL STAINS

Bharadwaj Bordoloi[1], Rohit Jaiswal[2], Safia Siddiqui[3], Rajeev Bhushan Singh[4]

[1]*Post Graduate student, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India, Pin code-226025*
[2]*Professor and Head, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India, Pin code-226025*
[3]*Senior Lecturer, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India, Pin code-226025*
[4]*Post Graduate student, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India, Pin code-226025*

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ABSTRACT: Special stains are dyes or substances used for special purpose in a histopathology laboratory. They help in differential coloration of cells and tissues in a specimen, help in visualization and thereby assist pathologists in diagnosis. These special stains have a long history of invention with the great efforts of the pioneer scientists and advent of new stain in line with the developments in the dye industry. This review assesses and compiles the current available literature to provide a sense of the rich legacy of histopathological analysis.

KEYWORDS: Special stain, dye, evolution, history

INTRODUCTION:

In any pathology, there are morphological changes in the cell, cell nucleus, and architectural changes in the extracellular matrix (ECM). For these changes to be detected in the tissue sections, the sections are stained with pigments[1]. A "stain" is any dye, reagent or other material used in colouring tissues or microorganisms for microscopical study. "Staining" is the artificial colouration of a substance to facilitate microscopic examination[2]. Histological staining is a series of technique processes undertaken in the preparation of sample tissues by using histological stains to aid in the microscope study[3]. The aim of staining is to reveal the cellular and ECM components and this forms the basis of histopathology[4]. Stains highlight important

Corresponding Author:
Bharadwaj Bordoloi,
Post Graduate student, Department of Oral Pathology, SPPGIDMS, Lucknow

features of the tissue as well as enhance the tissue contrast[5]. Histological staining is commonly used for pathological diagnosis and in forensic studies[6]. Proper staining of the sections, therefore, is of utmost importance for effective visualization of the tissue components and to establish an accurate diagnosis[7].

Hematoxylin is the cornerstone for staining tissue sections in routine histopathology[7] and the combination of hematoxylin and eosin (H&E) is the first stain applied to the all tissue sections and it gives critical diagnostic information in most cases[3]. But it cannot answer all the questions that a case might pose at the plain diagnostic level, and is clearly insufficient when one engages in an etiologic, histogenetic, or pathogenetic quest. As a consequence, the pathologist has always searched for additional techniques to probe those questions. Colloquially, these techniques have been referred to as 'special', simply because they are applied only under special circumstances[8].

The term "special stains" has long been used to refer to a large number of alternative staining techniques that are used when the H&E does not provide all the information the pathologist or researcher needs[3]. Special stains are not routinely used. The term "special stains" is of uncertain provenance, but one can be certain that it began to be used after 1876 when H&E was introduced[9].

Special stains have two broad areas of application: research and diagnostic. Special stains can be applied to cell biology and histology[9]. The aim of this review is to throw light upon the history of special stains and how they came into application in histopathology.

EVOLUTION OF SPECIAL STAINS:

Invention of microscope

Histology is the study of the microscopic details and structures of biological cells and tissues[10]. The first microscope had been constructed by Zacharias Jansen in 1591 in collaboration with his father in Holand but it had several optical problems[11]. In 1673 Anton van Leeuwenhoek developed a simple microscope with a single lens but with improved magnification and resolution[10]. In the mid-1800s, the invention of improved microscopes with corrected spherical and chromatic aberration by Ernst Abbe in Germany brought about a significant development in the field of histopathology[12]. The use of stains in the microscopic studies revolutionized the microscopic technique[13].

Other milestones

The early researchers used readily available laboratory chemicals such as potassium dichromate, mercuric chloride, and alcohol to harden the tissues so thin slices could be prepared for microscopical examination[12]. Formalin, which is a widely and universally used fixative today, was first employed in 1893[11,12,14]. Over the years different laboratory substances were

investigated for use as fixatives[10]. The earliest microscopists used free-hand sectioning that used to give poor results[12]. The first microtome suitable for sectioning animal tissues was constructed in 1848 and during the 19th century paraffin wax was introduced for infiltration and support during sectioning[10,12]. It was the introduction of the technique of embedding tissues in a solid medium and the use of dyes to enhance contrast and visibility that made histology into an accurate and reliable method[13].

Evolution of stains

Histological technique, as we know it today, seems to have become fairly well understood about 1860[13]. The pioneer scientists used naturally occurring substances such as madder, saffron, indigo, phytolacca to colour tissues, which they then studied under rudimentary microscopes[12]. Leeuwenhoek advocated the use of saffron in sections of muscle fibres to increase the visibility[11]. One of the oldest stains was Prussian blue, introduced in 1774[10]. Perls' reaction (1867), which uses Prussian blue for the histochemical localization of hemosiderin in tissues, is still widely used to localize intracellular iron. Picric acid, an important constituent of Bouin's fixative and Van Gieson's trichrome stain (1889), was discovered in 1788 and used as a yellow dye and disinfectant[15].

The introduction of stains into microscopic work has been ascribed to Hill (1770); Leeuwenhoek (1714), and Ehrenberg (1838)[13]. Joseph Von Gerlach was viewed as the pioneer of microscopical staining by many of his contemporaries, particularly in Germany. Gerlach, in 1858, reported in his paper the importance of staining (with carmine) in histology[5]. The earliest uses of stains were botanical, modern histological technique was first developed on zoological material. The first extensive use of stains was in animal histology[13].

Although natural dyes such as carmine and indigo were well known in the early days of the microscope, their use in staining microscopic preparations does not seem to have become common till about 1850[13]. Carmine was derived from the insect Coccus cacti. The use of carmine was documented in the reports of botanist John Hill in 1770. Rudolph Virchow (1821–1902), the "Father of Pathology," used carmine in his microscopy studies. Gerlach used ammoniacal carmine successfully to stain cerebellum cells. In 1896, Mayer introduced the mucicarmine stain by the addition of an aluminium mordant, while its modified techniques were introduced by Best and Southgate in 1906 and 1927, respectively. These stains were popular before alcian blue became available. The early microscopists also used metals such as silver nitrate to visualize tissue structure. They either rubbed solid silver nitrate into the tissue or immersed the tissue in a silver solution then studied the tissue microscopically[12].

Aniline (1856), the first of the synthetic dyes, was discovered by William Henry Perkin, while searching for a cure for

malaria[15]. The creation of the aniline dye industry in 1856 made significant impact with introduction of many new dyes some with applications in histopathology[12]. Hematoxylin, the routine stain in histopathology and a naturally occurring substance, was reportedly first used by Wilhelm von Waldeyer in 1863[7,12]. Different alum-based hematoxylins were introduced by Ehrlich in 1886, Harris in 1900, Mayer in 1903. Weigert introduced iron hematoxylin in 1904[7,12].

Robert Koch (1843–1910) established bacterial techniques to diagnose bacterial infections. In 1882, Robert Koch developed a method for the demonstration of the tubercle bacillus. He used various adaptations of the staining methods of Carl Weigert in smear microscopy. Later, several other researchers (Ehrlich, Ziehl, Rindfleisch, and Neelsen) introduced modifications to the original Koch's method and Ziehl-Neelsen stain and technique came into application[16]. In 1884, Hans Christian Gram introduced the Gram stain for identification of Gram positive organisms[17].

Hematology was revolutionized with the introduction in the 1890s the stains for blood smears[15]. Dimitri Romanowsky and Malachowski, in 1891, devised the popular stain for parasites in blood smears that is still widely used for this and other purposes today. Later Unna (1891), Jenner (1899), Lieshman (1901), Wright (1902), and Giemsa (1902) introduced the modified techniques[12].

Following the work of the pioneers in staining, the development of the subject was rapid, particularly after hematoxylin had been introduced by Waldeyer (1863) and more successfully by Bohmer (1865), aniline dyes by Beneke (1862) and alcohol differentiation by Bcittcher (1869)[13]. In the 19th century, histology was an eminent academic discipline in its own right[10]. During mid to late nineteenth century, the pathologists developed the intraoperative frozen section technique and adapted special stains techniques for use in histopathology[11]. Louis B. Wilson was the first to develop a method using methylene dyes to stain fresh-frozen tissue of surgical specimens (1906)[10].

The first half of the 20th century was a very productive period for new staining techniques in histology and histopathology[10]. Indeed the 1906 Nobel Prize in Physiology or Medicine was awarded to histologists Camillo Golgi and Santiago Ramon y Cajal for silver impregnation techniques for staining nerve tissue[10,15]. Many of the centenary staining techniques in cell biology and histopathology are still used and continue to provide valuable diagnostic information [10].

In animal histology, the early researchers started using multiple dyes in staining sections of animal tissue with an aim to differentiate nuclei from cell cytoplasm, to permit distinctions between the various types of tissue, and to have a better understanding of cell structure, function and the complex interrelationships between

elements within the tissue. These included haematoxylin with eosin Y, congo red, or safranin, various combinations of basic dyes such as crystal violet, methylene blue or one of the azures to stain the nuclei with some contrasting acid dyes to stain the cytoplasm of the cells[13]. With this, more and more cell and tissue elements were identified. Eventually, many protocols of differential staining, double staining or the multiple staining were developed, each targeting some particular element within the specimen[18].

It is, however, unclear when the term special stains first entered the histology/ pathology literature. An early documented use of the term can be found in the publication by Gomori in 1941. He used the term to describe a stain specifically created to differentially colour the insulin containing β-cells of the pancreas. In this case, "special" could be considered to be a "targeted" stain that is designed to identify a single cell or tissue constituent[18].

The first textbook of histology in the modern form appeared in the 1850s. Virchow published a medical journal which he edited for 50 years[11]. In 1959, Ann Preece published a textbook for histotechnicians. This text divided stains into three categories: Vital stains, Routine stains, and Special stains. As per the definitions in that text, special stains are those that have a "more limited range" and that demonstrate special features. Cited examples included bacteria, fungi, particular cell products and microscopic intracellular and intercellular products. The definition provided by Preece is in some ways carried over to current usage, but is not totally accurate. Preece's definition for "routine stain" is much broader than the current usage, according to which, all of the connective tissue stains, such as reticulum and trichromes, would be considered as "routine". In the modern histopathology laboratory, H&E is referred to as the "Gold Standard", and is the first stain performed on almost all specimens. All subsequent stains fall under the definition of "special stains". There is one exception to this, and that is the immunohistochemical stains (IHC). Although IHC stains meet every criteria of the definition of "special stains" the FDA specifically excluded them from this category when they first regulated IHC stains[18].

During 1950s and 1960s, discoveries in histochemistry, cytochemistry and autoradiography prospered. The transmission electron microscope, introduced in the 1960s, brought about significant discoveries in cell ultrastructure and functions of cell organelles[19]. As understanding has continued to develop, cell biology has expanded into the realm of molecular biology. The foundations of cell and molecular biology were generated by stain protocols, many of which were the "special stains" still in use today[18].

Special stains, as currently defined in diagnostic pathology, consist of several types of stains[18]. Dr. Juan Rosai, the well-known pathologist categorized the special stains, he

used in his laboratory, into 14 groups as periodic acid-Schiff (PAS), organisms stains, argentaffin and argyrophilic stains, amyloid stains, reticulin stains, trichrome stains, phosphotungstic acid hematoxylin, stains for melanin, calcium and iron, stains for neutral lipids, mucin stains, Giemsa stains, elastic stains, myelin stains, and formaldehyde-induced fluorescence[8].

Although some special stains were derived from histochemical investigations, many were developed strictly as morphological stains, i.e., the stains which demonstrate some particular morphology, e.g., stains for microorganisms, for myelin and nerve fibres, and for connective tissues including reticular fibres. Special stains for specific tissue components (mainly histochemical) are stains for iron, mucins and glycogen, amyloid, and nucleic acids[18].

IHC stains have replaced many traditional special stains simply because they have great specificity and ability to recognize precisely the target or epitope[18]. But the special stains still play an important role in surgical pathology and some at this time are irreplaceable, such as the trichrome methods for renal and liver biopsies and silver nitrate methods for organisms[12]. Some special stains are also exquisitely sensitive, for example, the iron stain actually detects ions of a single element, the PAS stain detects exceptionally small amounts of glycogen and mucopolysaccharides, the Feulgen reaction can detect DNA accurately enough to detect the gain or loss of a single one of the larger chromosomes (the basis of Ploidy measurements)[18]. IHC staining is not suitable for identification of elemental inclusions, such as iron. IHC tests are expensive, and may not be readily available in all laboratories[18].

In the modern age of histology there have been significant improvements in histological stains and techniques. A few modern stains used are Masson's Trichrome for connective tissues, Golgi stain for neuronal fibres, Toluidine Blue for mast cells and as vital stain, Kluver-Barrera stain for Lipofuscin, Mallory's CT stain, PAS for glycogen[5]. The modern practice of pathology depends on both the special stains and IHC stains. Histological techniques have not altered as much in the past century as those of other scientific disciplines. Significant change in microscopic diagnosis will certainly generate changes in the use of special stains and there will always be a need for special stains for bacteria, fungi, iron, and general tissue architecture[18].

CONCLUSION:

The pioneers in histopathology have made great contribution by discovering the stains for coloring of tissues. Though many stains have been replaced with IHC because of the complex staining procedures or the stains being harmful, many other stains are still very popular and are in use. While compared to IHC, it should also be kept in mind, that special stain procedures also offer a cost-effective alternative and many such staining procedures can be performed with limited resources at a simple laboratory set

up. The efforts of pioneer scientists and the gradual advent of different stains and staining techniques makes the history of histopathology very interesting and informative and one must have a knowledge on this to have a good understanding of the discipline.

REFERENCES:

1. Ross MH, Pawlina W. Histology-A Text and Atlas. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
2. Dorland's Illustrated Medical Dictionary. 28th ed. New Delhi: Harcourt Brace & Co. Asia PTE Ltd; 1994.
3. Anderson J. An introduction of routine and special staining. Germany: Leica Biosystems; 2011. Available from: www.leicabiosystems.com/pathologyleaders [Last accessed on 2017 Sep 01]
4. Black JG. Microbiology-principles and exploration. 8th ed. New Jersey: John Wiley & Sons; 2012.
5. Titford M. The long history of haematoxylin. Biotech Histochem. 2005;80(2):73-8.
6. Gurcan MN, Boucheron L, Can A, Madabhushi A, Rajpoot N, Yener B. Histopathological image analysis-a review. IEEE Rev Biomed Eng. 2009; 2(1):147-71.
7. Allison RT. Haematoxylin-from the wood. J Clin Pathol. 1999;52:527-8.
8. Rosai J. Rosai and Ackerman's Surgical Pathology. 10th ed. London: Mosby; 2011.
9. Kumar GL, Gill GW. Introduction to Special Stains. In Kumar GL, Kiernan JA (ed). Dako Education guide-special stains and H&E. 2nd ed. California: Dako; 2010. Available from: www.dako.com [Last accessed on 2017 Sep 01].
10. Bordoloi B, Jaiswal R, Siddiqui S, Tandon A. Health Hazards of Special Stains. Saudi J. Pathol. Microbiol. 2017;2(5):175-8.
11. Musumeci G. Past, present and future: overview on histology and histopathology. J Histol Histopathol. 2014; 1(5):1-3.
12. Titford M. A short history of histopathology technique: J Histotechnol. 2006; 29(2):99-110.
13. Conn HJ. Biological stains; a handbook on the nature and uses of the dyes employed in the biological laboratory, 6th ed. Maryland: The Williams & Wilkins Company; 1953.
14. Cook DJ. Cellular Pathology – An Introduction to Techniques and Applications. 2nd ed. Oxfordshire: Scion Publishing Ltd.; 2006.
15. Titford M. Progress in the development of microscopical techniques for diagnostic pathology. J Histotechnol. 2009;32(1):9-19.
16. Titford M and Bowman B. What May the Future Hold for Histotechnologists? LabMedicine. 2012;43:5-10.
17. Costa CAX, Brito KNO, Gomes MA, Caliari MV. Histopathological and immunohistochemical study of the hepatic lesions experimentally induced by Entamoeba. Eur. J. Histochem. 2010;54(3):170-4.
18. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological Stains-a literature review and case study. GJHS. 2016;8(3):72-9.
19. Bishop PJ, Neumann G. The history of the Ziehl–Neelsen stain. Tubercle. 1970;51:196-206.

20. Bartholomew JW, Mittwer T. The Gram stain. Bacteriol Rev. 1952;16(1):1-29.
21. Rodrigues EB, Costa EF, Penha FM, Melo GB, Bottós J, Dib E, Farah ME. The use of vital dyes in ocular surgery. Surv Ophthalmol. 2009; 54(5):576-617.
22. Alton D. Evolution of use of special stains. In Kumar GL, Kiernan JA (ed). Dako Education guide-special stains and H&E. 2nd ed. California: Dako; 2010. Available from: www.dako.com [Last accessed on 2017 Sep 01].
23. Horobin RW, Kiernan JA. Conn's Biological Stains: A Handbook of Dyes and Fluorochromes for Use in Biology and Medicine. 10th ed. Oxford: BIOS Scientific Publishers; 2002.

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