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COMPARISON OF THREE ALUM HEMATOXYLIN – HARRIS, MAYER'S, EHRLICH HAEMATOXYLIN USING PROGRESSIVE & REGRESSIVE METHOD – A STUDY OF 60 CASES

Oral Pathology

Dr Varun Rastogi* Professor, Department of Oral & Maxillofacial Pathology, UCMS, Ranigaun, Bhairahawa, Nepal *Corresponding Author

Dr Nisha Maddheshiya Lecturer, Department of Oral Medicine & Radiology, UCMS, Ranigaun, Bhairahawa, Nepal

ABSTRACT

Background: The contribution of staining techniques in brightfield microscopy has been remarkable, considering that many of these staining techniques are still widely used for diagnostic purposes more than a century after their introduction. Each working day in laboratories around the world, millions of microscope slides stained with Hematoxylin and Eosin are prepared and viewed by pathologists as part of the diagnostic process.
Materials and Methods: The study included histological sections of four groups and 3 hematoxylin solutions. Each group of hematoxylin had sections of four groups. Hence, a total number of 60 sections were made.
Results: The analysis of the relationship of the following variables, namely, different alum hematoxylin concluded that Harri's hematoxylin was superior to Mayer's and Ehrlich hematoxylin.
Conclusion: Our study has shown much promise in exploring Hematoxylin and Eosin stain as a routine staining procedure. The persistence and continuing viability and growth of Hematoxylin and Eosin morphology indicates that this simple technique continues to meet most of the requirements of not only the pathologists but also clinicians, and, let us not forget, patients.

KEYWORDS

Alum Hematoxylin, Harri's hematoxylin, Mayer's hematoxylin, Ehrlich's hematoxylin.

INTRODUCTION

It is commonplace, virtually platitudinous to say that the practice of histopathology has changed almost beyond recognition during the last three or four decades. It is correct that the scope of pathology has widened and that greater diagnostic accuracy can often be achieved. (1) There is probably no other area in cell biology where simple histological techniques have survived, many in their original form, from a period before the current generation of cell biologists were born. (2)

In these days of rapidly advancing laboratory technology, the most commonly used stain in biology is based on hematoxylin, a naturally occurring compound derived from the logwood tree hematoxylin campechianum (3).

The Hematoxylin and Eosin stain is a special nuclear stain which is routinely performed in histology laboratories. The stain theory is based on the attraction of opposite charged tissue and dye molecules. (4) The combination of mordant and dye is known as a 'lake' and in the case on hematoxylin – mordant such lake is positively charged, behaving as cationic dyes at low pH (5). Numerous histological and histochemical staining solutions use hematoxylin together with mordanting metals such as aluminium, chromium, iron, tungsten, lead and molybdenum. (6)

Hematoxylin can be used as either a progressive or regressive stain. In progressive staining, a milder form of hematoxylin is used that will only stain the nucleus of the cell and cause the nuclear materials to turn a deeper blue when rinsed in water eg. Gill's, Mayer's Hematoxylin. In regressive staining a stronger form of hematoxylin is used that will stain everything on the slide and holds fast to the tissue when rinsed. (4) The color shifts from blue / purple to salmon pink / red. eg. Harri's, Ehrlich's hematoxylin.

Hematoxylin can be ripened either by natural process by exposure to air and light and takes 3 – 4 months eg. Ehrlich's and Delafield hematoxylin or by chemical oxidation using sodium iodate e.g. Mayer's hematoxylin or mercuric oxide eg. Harri's hematoxylin. The use of chemical oxidizing agents converts hematoxylin to hematein almost instantaneously, so these hematoxylin solutions are ready to use immediately after preparation. (7)

The aim of the study was to visualize and compare different alum hematoxylin and its histopathological picture, the clarity of staining and retention of stain inside the various tissues.

MATERIALS AND METHODS

Materials

The study included histological sections of four groups – Type I (well differentiated Oral squamous cell carcinoma), II (normal Lymph node), III (normal mucous acini), IV (Fibroepithelial hyperplasia). The tissue block was retrieved from the archives of department of Oral and Maxillofacial Pathology, Saveetha Dental College, Chennai India from 2005 onwards. The sections were cut using Leica Semi-automatic microtome (RM2245) and the thickness of sections was 3 microns. The study comprised of 3 solutions – solution 1 (Harri's hematoxylin), 2 (Mayer's hematoxylin), 3 (Ehrlich's hematoxylin). Each group of hematoxylin had sections of four groups and stained using progressive or regressive method. Hence, a total number of 60 sections were made.

METHODOLOGY

Preparation of staining solution

1. Ehrlich's hematoxylin (8, 9): Dissolve 2g of hematoxylin in 100ml of absolute alcohol. Then 100ml of distilled water was added, 10ml of glacial acetic acid and 15g of potassium alum with constant stirring. 100ml of glycerine was added to the oxidation process and prolong the hematoxylin shelf life. Natural ripening in sunlight takes about 2 months.

2. Mayer's Hematoxylin (10, 11): Dissolve 1g of hematoxylin with 50g potassium alum and 0.2g sodium iodate in 1000 ml distilled water by warming and stirring, or by allowing standing at room temperature overnight. 50g chloral hydrate and 1g citric acid were then added, and the mixture was boiled for 5min, then cooled and filtered.

3. Harri's Hematoxylin (12): 2.5g hematoxylin was dissolved in 25ml absolute alcohol, and was then added to 50g potassium alum, which has been dissolved in the warm 500ml distilled water in a 2-litre flask. The mixture is rapidly brought to the boil and 125g mercuric oxide or 0.5g sodium iodate is then slowly and carefully added. Plunging the flask into cold water or into a sink containing chipped ice rapidly cools the stain. When the solution was cold, 20ml glacial acetic acid was added, and the stain was ready for immediate use.

Procedure: Hematoxylin staining

- Dewax the section, hydrated through graded alcohol to water
- Remove fixation pigments
- Harri's hematoxylin – 5-15 min
- Mayer's hematoxylin – 5 -10 min
- Ehrlich's hematoxylin – 30 min – 1hr
- Wash well in running tap water until section blue for 5min
- Differentiate in 1 acid alcohol for 5-10sec
- Wash well in tap water
- Blue by dipping in an alkali solution (ammonia water) followed by 5 min tap water wash

- Stain in 1 Eosin Y for 1 min
- Wash in running tap water for 1-5min
- Dehydrate through alcohols, clear and mount

All the stained sections were assessed and tabulated. The assessment parameters included are as follows: Epithelium cell membrane, nucleus and cytoplasm staining of stratum basale, spinosum, granulosum and corneum layer were assessed. Connective tissue – collagen fibres, fibroblasts, inflammatory cells, adipocytes, blood vessels, muscle and gland were assessed.

The scoring criteria used to grade the intensity of tissue staining as follows:
+++ --> 3, ++ --> 2, + --> 1, - -->-0

Mean scores were estimated from the sample for each study groups. Mean scores were compared between different groups by using either by Mann-Whitney U test α Kruskal Wallis One Way ANOVA followed by Mann-Whitney U test. In the present study, $P<0.05$ was considered as the level of significance. The statistical analysis software used in the study is SPSS version 13.

RESULTS

As per **Table 1, 2, 3, 4** Comparison of mean scores between different solutions in type I - IV group indicates that Harri's hematoxylin is superior to Mayer's and Ehrlich hematoxylin. This is proved further by statistical test (Kruskal – Wallis one-way ANOVA and Mann-Whitney U test) which showed that there is a significant difference in mean among the three solution ($P<0.05$).

Table 5, 6, 7, 8 shows that there is no significant difference between progressive and regressive staining method.

Table 1: Comparison of mean scores between different solution in Type I group (oral squamous cell carcinoma).

Variable	Solution	Mean± S.D.	P-Value **	Significant # groups at 5% level
CM1	I II III	2.6±0.7 2.2±0.3 1.5±0.6	0.04	I Vs III II Vs III
N1	I II III	2.5±0.7 2.2±0.3 1.5±0.6	0.06	NIL
C1	I II III	2.6±0.7 2.2±0.3 1.3±0.3	0.009	I Vs III
CM2	I II III	2.1±0.4 1.3±0.4 1.1±0.2	0.01	I Vs II I Vs III
N2	I II III	2.0±0.5 1.3±0.4 1.1±0.2	0.02	I Vs II I Vs III
C2	I II III	2.1±0.4 1.3±0.4 1.1±0.2	0.01	I Vs II I Vs III
CM3	I II III	1.7±0.6 1.0±0.0 1.0±0.0	0.007	I Vs II I Vs III
N3	I II III	1.9±0.7 1.0±0.0 1.0±0.0	0.007	I Vs II II Vs III
C3	I II III	1.8±0.8 1.0±0.0 1.0±0.0	0.007	I Vs II I Vs III
CM4	I II III	2.3±0.4 1.4±0.5 1.0±0.0	0.007	I Vs II I Vs III
N4	I II III	2.2±0.6 1.4±0.5 1.0±0.0	0.01	I Vs III
C4	I II III	2.3±0.4 1.4±0.5 1.0±0.0	0.007	I Vs II I Vs III
CT1	I II III	2.3±0.3 1.4±0.5 1.4±0.4	0.02	I Vs II I Vs III
CT2	I II III	2.3±0.3 1.0. ±0.0 1.3±0.3	0.003	I Vs II I Vs III II Vs III
CT3	I II III	2.5±0.4 1.8±0.4 1.3±0.3	0.006	I Vs II I Vs III
CT4	I II III	1.5±0.7 0.6±0.5 0.0±0.0	0.008	I Vs III II Vs III
CT5	I II III	2.5±0.5 1.2±0.4 1.4±0.2	0.008	I Vs II I Vs III
CT6	I II III	2.1±0.7 0.6±0.5 0.6±0.8	0.02	I Vs II I Vs III
CT7	I II III	0.9±1.3 0.4±0.5 0.3±0.7	0.72	NIL

Table 2: Comparison of mean score among different solutions in Type II group lymph node).

Variable	Solutions	Mean±S.D	P-Value**	Significant# groups at 5% level
CT1	I II III	1.5±0.7 1.2±0.4 1.0±0.0	0.42	NIL
CT2	I II III	1.7±0.6 1.2±0.3 1.0±0.0	0.03	I Vs III
CT3	I II III	2.5±0.5 1.7±0.3 1.2±0.4	0.009	I Vs II I Vs III
CT4	I II III	1.7±0.4 1.1±0.2 1.0±0.0	0.004	I Vs II I Vs III
CT5	I II III	2.0±0.5 1.4±0.2 1.0±0.0	0.004	I Vs II I Vs III II Vs III
CT6	I II III	0.6±0.2 0.5±0.0 0.9±0.2	0.03	II Vs III
CT7	I II III	0.0±0.0 0.0±0.0 0.0±0.0	1.00	NIL

Table 3: Comparison of mean scores among different solutions in Type III group (Gland)

Variable	Solution	Mean± S.D	P – Value	Significant # groups at 5% less
CM1	I II III	1.7±0.6 1.9±0.2 1.4±0.2	0.09	NIL
N1	I II III	1.9±0.5 1.6±0.2 1.0±0.0	0.004	I Vs III II Vs III
C1	I II III	1.9±0.7 1.7±0.3 1.6±0.2	0.66	NIL
CM2	I II III	1.8±0.4 1.5±0.4 1.5±0.4	0.46	NIL
N2	I II III	1.9±0.7 1.4±0.4 1.2±0.3	0.17	NIL
C2	I II III	1.8±0.7 1.4±0.2 1.5±0.5	0.60	NIL
CT1	I II III	1.8±0.4 1.3±0.3 1.0±0.0	0.02	I Vs III II Vs III
CT2	I II III	1.6±0.5 1.4±0.4 1.2±0.3	0.41	NIL
CT3	I II III	0.7±0.4 0.7±0.3 1.0±0.4	0.35	NIL

Ct4	I II III	1.0±0.8 0.9±0.2 1.2±0.3	0.46	NIL
CT5	I II III	1.7±0.4 1.4±0.4 1.5±0.4	0.47	NIL
CT6	I II III	0.0±0.0 0.0±0.0 0.0±0.0	1.00	NIL
CT7	I II III	0.0±0.0 0.0±0.0 0.0±0.0	1.00	NIL

Table 4: Comparison of mean scores among different solutions in Type 4 group (fibroepithelial hyperplasia).

Variable	Solution	Mean±S.D	P-Value **	Significant # groups at 5% level
CM1	I II III	2.5±0.5 1.8±0.5 1.2±0.4	0.01	I Vs II I Vs III
N1	I II III	2.6±0.4 1.7±0.4 1.3±0.4	0.009	I Vs II I Vs III
C1	I II III	2.5±0.5 1.8±0.4 1.2±0.4	0.01	I Vs II I Vs III
CM2	I II III	1.6±0.4 1.0±0.0 1.1±0.2	0.02	I Vs II I Vs III
N2	I II III	1.6±0.4 1.0±0.0 1.2±0.3	0.03	I Vs II
C2	I II III	1.6±0.4 1.0±0.0 1.1±0.2	0.02	I Vs II I Vs III
CM3	I II III	1.5±0.5 1.0±0.0 1.1±0.2	0.09	NIL
N3	I II III	2.0±0.4 1.1±0.2 1.2±0.3	0.008	I Vs II I Vs III
C3	I II III	1.5±0.5 1.0±0.0 1.1±0.2	0.09	NIL
CM4	I II III	1.5±0.5 1.0±0.0 1.2±0.4	0.13	NIL
N4	I II III	1.7±0.6 1.0±0.0 1.1±0.2	0.02	I Vs II
C4	I II III	1.5±0.5 1.0±0.0 1.2±0.4	0.13	NIL
CT1	I II III	2.0±0.0 1.0±0.0 1.4±0.5	0.009	I Vs II I Vs III
CT2	I II III	2.1±0.4 1.1±0.2 1.3±0.4	0.01	I Vs II I Vs III
CT3	I II III	2.5±0.4 1.8±0.3 1.6±0.2	0.009	I Vs II I Vs III
CT4	I II III	0.0±0.0 0.0±0.0 0.1±0.2	0.37	NIL
CT5	I II III	2.2±0.4 1.0±0.0 1.5±0.4	0.004	I Vs II I Vs III II Vs III
CT6	I II III	0.5±0.7 0.5±0.0 1.0±0.7	0.31	NIL
CT7	I II III	0.0±0.0 0.0±0.0 0.0±0.0	1.00	NIL

Table 5: Mean standard deviation and test of significance of mean scores between 'Progressive' and method 'Regressive' using different solutions & Type I group (oral squamous cell carcinoma).

Variable	Method – P Mean ± S.D.	Method – R Mean ± S.D.	P- Value *
CM1 N1 C1	2.6±0.7 2.5±0.7 2.6±0.7	2.9±0.2 2.9±0.2 2.9±0.2	0.44 0.37 0.44
CM2 N2 C2	2.1±0.4 2.0±0.5 2.1±0.4	2.4±0.2 2.4±0.2 2.2±0.4	0.19 0.15 0.65
CM3 N3 C4	1.7±0.4 1.9±0.7 1.8±0.8	1.7±0.4 1.7±0.4 1.7±0.4	1.00 0.58 0.91
CM4 N4 C4	2.3±0.4 2.2±0.6 2.3±0.4	2.4±0.4 2.4±0.4 2.4±0.4	0.65 0.51 0.65
CT1 CT2 CT3 CT4 CT5 CT6 CT7	2.3±0.3 2.3±0.3 2.5±0.4 1.5±0.7 2.5±0.5 2.1±0.7 0.9±1.3	2.2±0.3 2.2±0.4 2.3±0.3 1.2±1.0 2.5±0.4 1.5±1.1 0.5±1.1	0.55 0.40 0.34 0.52 1.00 0.34 0.52

Table 6: Mean standard deviation and test of significance of mean scores between 'Progressive' and method 'Regressive' using different solutions & Type II group (lymph node).

Solution	Variable	Method –P Mean ±S.D	Method –R Mean ±S.D	P- Value *
I	CT1 CT2 CT3 CT4 CT5 CT6 CT7	1.5±0.7 1.7±0.6 2.5±0.5 1.7±0.4 2.0±0.5 0.6±0.2 0.0±0.0	1.9±0.2 2.2±0.4 2.7±0.4 1.6±0.7 2.1±0.4 0.9±0.2 0.0±0.0	0.37 0.15 0.50 0.73 0.74 0.07 1.00
II	CT1 CT2 CT3 CT4 CT5 CT6 CT7	1.2±0.4 1.2±0.3 1.7±0.3 1.1±0.2 1.4±0.2 0.5±0.0 0.0±0.0	1.8±0.4 1.5±0.4 2.1±0.7 1.2±0.6 1.3±0.4 0.5±0.0 0.0±0.0	0.07 0.17 0.31 0.81 0.49 1.00 1.00
III	CT1 CT2 CT3 CT4 CT5 CT6 CT7	1.0±0.0 1.0±0.0 1.2±0.4 1.0±0.0 1.0±0.0 0.9±0.2 0.0±0.0	1.0±0.0 1.0±0.0 1.6±0.2 1.0±0.0 1.0±0.0 1.0±0.0 0.0±0.0	1.00 1.00 0.07 1.00 1.00 0.32 1.00

Table 7: Mean standard deviation and test of significance of mean scores between 'Progressive' and method 'Regressive' using different solutions & Type III group (Gland)

Solution	Variable	Method – P Mean ± S.D	Method – R Mean ± S.D	P – Value *
I	CM1 N1 C1	1.7±0.6 1.9±0.5 1.9±0.7	2.4±0.5 2.3±0.6 2.3±0.6	0.08 0.27 0.34
	CM2 N2 C2	1.8±0.4 1.9±0.7 1.8±.07	2.3±0.3 2.2±0.3 2.1±0.2	0.08 0.31 0.44
	CT1 CT2 CT3 CT4 CT5 CT6 CT7	1.8±0.4 1.6±0.5 0.7±0.4 1.0±0.8 1.7±0.4 0.0±0.0 0.0±0.0	1.6±0.5 2.1±0.2 1.1±0.2 1.0±0.9 2.0±0.4 0.0±0.0 0.0±0.0	0.51 0.09 0.09 0.92 0.29 1.00 1.00
II	CM1 N1 C1	1.9±0.2 1.6±0.2 1.7±0.3	1.7±0.6 1.3±0.3 1.5±0.6	0.43 0.09 0.31
	CM2 N2 C2	1.5±0.4 1.4±0.4 1.4±0.2	1.4±0.2 1.3±0.3 1.0±0.0	0.61 0.73 0.01

	CT1	1.3±0.3	1.0±0.0	0.0495
	CT2	1.4±0.4	1.3±0.3	0.73
	CT3	0.7±0.3	0.8±0.3	0.55
II	CT4	0.09±0.2	0.6±0.4	0.19
	CT5	1.4±0.4	1.3±0.3	0.73
	CT6	0.0±0.0	0.0±0.0	1.00
	CT7	0.0±0.0	0.0±0.0	1.00
III	CM1	1.4±0.2	1.6±0.7	0.73
	N1	1.0±0.0	1.4±.07	0.14
	C1	1.6±0.2	2.0±0.5	0.15
	CM2	1.5±0.4	1.6±0.7	0.91
	N2	1.2±0.3	1.5±0.7	0.64
	C2	1.5±0.5	2.0±0.5	0.16
	CT1	1.0±0.0	1.2±0.3	0.13
	CT2	1.2±0.3	1.5±0.5	0.31
	CT3	1.0±0.4	1.1±0.4	0.65
	CT4	1.2±0.3	1.4±0.4	0.42
	CT5	1.5±0.4	1.8±0.7	0.50
	CT6	0.0±0.0	0.0±0.0	1.00
	CT7	0.0±0.0	0.0±0.0	1.00

Table 8: Mean standard deviation and test of significance of mean scores between 'Progressive' and method 'Regressive' using different solutions & Type IV group (fibroepithelial hyperplasia).

Variable	Method – P Mean ± S.D.	Method – R Mean ± S.D.	P – Value *
CM1	2.5±0.5	2.9±0.2	0.15
N1	2.6±0.4	2.9±0.2	0.19
C1	2.5±0.5	2.9±.02	0.15
CM2	1.6±0.4	2.0±0.7	0.26
N2	1.6±0.4	2.1±0.4	0.10
C2	1.6±0.4	2.0±0.7	0.26
CM3	1.5±0.5	1.9±0.5	0.21
N3	2.0±0.4	2.3±0.4	0.17
C3	1.5±0.5	1.9±0.5	0.21
CM4	1.5±0.5	1.9±0.5	0.21
N4	1.7±0.6	2.3±0.4	0.09
C4	1.5±0.5	2.3±0.6	0.15
CT1	2.0±0.0	2.2±.04	0.32
CT2	2.1±0.4	2.4±0.2	0.19
CT3	2.5±0.4	2.7±0.3	0.34
CT4	0.0±0.0	0.0±0.0	1.00
CT5	2.2±0.4	2.4±0.4	0.57
CT6	0.5±0.7	0.4±0.5	0.81
CT7	0.0±0.0	0.0±0.0	1.00

CM1 - Cell Membrane in Basal layer; CM2 - Cell Membrane in Spinosum layer; CM3 - Cell Membrane in Granular layer; CM4 - Cell Membrane in Corneum layer

N1 - Nucleus in Basal layer; N2 - Nucleus in Spinosum layer; N3 - Nucleus in Granular layer; N4 - (Cell Membrane in Corneum layer
C1 - Cytoplasm in Basal layer; C2 - Cytoplasm in Spinosum layer; C3 - Cytoplasm in Granular layer; C4 - Cell Membrane in Corneum layer
CT1 - Collagen fibers; CT2 - Fibroblast
CT3 - Inflammatory Cells; CT4 - Adipocytes
CT5 - Blood vessels; CT6 - Muscle
CT7 - salivary gland

DISCUSSION

Staining is defined as the visual labelling of some entity by attaching or depositing in its vicinity, a marker of contrast color and shape. Successful histological techniques used for the distinction of tissue components commonly cause two changes in the tissue, either an alteration of contrast or an alteration in colour.

Hematoxylin is a naturally occurring chemical used as the basis of dye in laboratories throughout the world to stain nuclei in microscope slide preparation. This chemical is extracted from the logwood tree hematoxylin campechianum a tree of the order leguminosae (genus eucaesalpineae) and so named because of the reddish color of its heartwood (from the Greek Heamoto – blood xylon – wood) and young leaves. (13) The heartwood is very hard and heavy and may range from dark orange to puplish red. (14). The crude logwood product also contains tannins, resins, quercitin and a small amount of volatile oil (15).

The basic principle involves oxidation of hematoxylin to hematein, which is anionic form, hence cannot stain the tissue. Hematein is then combined with a mordant to convert anionic form to cationic form which ultimately results in staining of tissues. This oxidation of hematoxylin to hematein can occur naturally by exposure to air and light or can be done using chemical agents such as sodium iodate, mercuric oxide etc.

Marshall and Horobin 1972 found that oxyhematein is a carboxylic acid and at least one break in the linkage region between the aromatic and quinonoid ring must occur during the oxidation of hematein. This oxyhematein gives an orange yellow colour to the tissues (17, 18). The affinity of the stain to the tissue depends upon the concentration of the dye and the amount of mordant in the staining solution. If the mordant is more than the dye in the staining solution, the stain will bind firmly to the tissues and the bond between the stain and tissues cannot be broken easily. If the amount of dye is more than the mordant, the stain binds loosely to the tissues and the bond between the stain and tissue is easily broken.

Baker showed that in case of mordant dyeing such as with alum hematoxylin, the acid disrupts the tissue mordant bond rather than the mordant dye bond. (19) It is often remarked that the addition of a mordant to an appropriately dye solution result in a very sudden, dramatic change in color. This is due to the incorporation of metal atom into the delocalized electron system of the dye. When a mordant is used in conjunction with a dye to stain the tissues, the mordant may also be used to remove excess dye.

DNA, RNA, and phospholipids are acidic due to their phosphoryl groups, and mast cells, cartilage, and some mucous secretions of glands contain acidic sulphuryl and carboxyl groups. Collagen, Red blood corpuscles, and the granules of eosinophil, leucocytes are basic due to the predominance of basic amino groups. (20) The attachment of the mordant metal to tissue is by chelation – covalent and coordinate bond formation and the attachment of the mordant dye is due to the mordant forming a chelate with the phosphate hydroxyl and another atom in a manner very similar to that between the mordant and the dye. Electrostatic attractions are probably important in pulling dye molecules towards oppositively charged parts of tissue. When staining is by a dilute solution of cationic or anionic dyes with small molecules, ionic bonds may be the only forces holding dye to substrate. (21)

Marshall and Horobin 1972 suggested that dye-metal complexes are bound to chromatin by both ionic and non - ionic forces. (22) The latter are likely to be enhanced by the other substance present in alum-hematein staining solutions. Most formulations contain a highly polar substance such as glycerol, ethylene glycol or chloral hydrate, which would be expected to associate by hydrogen bonding with hydrophilic components of the tissue and to interfere with short range forces (Vander walls, hydrophobic etc) that would hold the dye-metal complex to some potential substrates.

The reactions of aluminium ions with hematein have been studied by **Bettinger and Zimmermann (1991)** who found that a cationic dye – metal complex was present in acid solution. (23, 24) The complex was bound by DNA in section of tissue, even though the pH was lower than that at which nucleic acids can be stained by ordinary cationic dyes. The hemalum mixtures in common use contain a large excess of Al3+ ions over hematein molecules. Aluminium ions have considerable affinity for DNA and can prevent its subsequent staining by basic dyes. Acids used to increase the selectively of nuclear staining probably disrupt the bonding between Al^{3+} and parts of tissues other than chromatin, rather than between Al3+ and the dye.

CONCLUSION

Our study has shown much promise in exploring Hematoxylin and Eosin stain as a routine staining procedure. Further studies on Hematoxylin and Eosin could open a new horizon in the broad field of laboratory techniques. The persistence and continuing viability and growth of Hematoxylin and Eosin morphology indicates that this simple technique continues to meet most of the requirements of not only the pathologists but also clinicians, and, let us not forget, patients.

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