USEFULNESS OF METACHROMATIC REAGENTS IN
DISCRIMINATING BETWEEN CONNECTIVE TISSUE AND
EPITHELIAL MUCOPOLYSACCHARIDES

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ABSTRACT

The present study compares twelve different histochemical methods of demonstrating mucopolysaccharides in normal and diseased tissues. The metachromatic dyes (Azur A and Thionin B) were the most useful stains for distinguishing epithelial mucopolysaccharides, which were orthochromatic, from connective tissue mucopolysaccharides, which were metachromatic. Periodic acid-Schiff, Alcian blue, Southgate mucicarmine, and Hale colloidal iron stains were useful for the demonstration of mucopolysaccharides in general. However, these stains did not aid in the discrimination of epithelial from connective tissue mucopolysaccharides. Hexamine silver, Acrifine orange and blocking of reactive groups by methyl esterification, and saponification are technically inferior and not useful for demonstration of mucopolysaccharides.

Keywords: Metachromatic dyes, Mucopolysaccharides, Tissue processing.

INTRODUCTION

Mucosubstances include mucopolysaccharides and their complexes with proteins and lipids (Stacey and Barker, 1962). There are three main types of mucosubstances, namely, mucopolysaccharides (hexoses and hexosamine polymers), mucoproteins (complexes of proteins and mucopolysaccharides) and mucolipids (complexes of lipid and mucopolysaccharides).

Mucoproteins are also referred to as mucins and may be further classified as acidic or neutral (Troyer, 1960). Acidic mucoproteins possess carboxylated glucose units such as in hyaluronic acid, or sulphated glucosamine units such as in heparan and chondroitin. Neutral mucoproteins do not contain these acidic carboxyl or sulphate radicals (Troyer, 1960).

Mucosubstances have a widespread distribution and complex staining reaction (Walter and Israel, 1987). They occur in the lining epithelia and secretions of the respiratory and gastrointestinal tracts as well as in the ground substance of various connective tissues, where they are produced in the Golgi bodies of fibroblasts, osteoblasts, chondroblasts, and mast cells. Various mucopolysaccharides are also provided by breast, urinary, ovarian, pancreatic, thyroid and intestinal cancer cells (Walter and Israel, 1987).

The demonstration and characterization of mucosubstances is of practical importance in the illustration of normal historical structures in the diagnosis of certain pathological lesions, including for example, salivary gland and colonic neoplasms.

MATERIALS AND METHODS

The materials for the present study were normal and diseased tissues selected from surgical and postmortem biopsy specimens received in the Department of Pathology, University College Hospital, Ibadan. The normal tissues were colonic mucosa, lung, bronchial cartilage and mucosal glands) submandibular salivary gland, endocervical invasive ductal mammary carcinoma, colonic adenocarcinoma, mucinous cystadenoma of the ovary, chondrosarcoma, pleomorphic adenoma, and mucinous salivary gland cyst.

The tissues were fixed in formalin and in a distillate of raffia palm wine gin (ufolob) (Umoh, 1995) for at least 20 hours before tissue processing. The surgical specimens were processed routinely by dehydration in graded concentration of native gin, obtained from the distillation of raffia palm wine, clearing in a 70:30 mixture of palm kernel oil and xylene (Umoh, 1995), followed by infiltration and embedding in paraffin wax.

All the above-mentioned tissues were subjected to the following stains.

1. PERIODIC ACID SCHIFF, WITH AND WITHOUT DIASTASE

Preparation of Solutions

1% aqueous periodic acid
Schiff’s reagent:— Add Ig of basic fuchsin to 200ml of boiling distilled water and dissolve. Cool to 50°C and 2g of Potassium meta bisulphite. Dissolve, allow cooling to room temperature and add 2mls of concentrated Hydrochloric acid. Leave overnight in the dark, and then add 012g of activated charcoal and shake for 1-2 minutes. Filter and store in a dark brown bottle, ready for use.

Methods
De-wax sections and take down to water followed by washing in distilled water. Treat with periodic add solution for 2 minutes. Rinse in distilled water and treat with Schiff reagent for 8 minutes. Wash in running water for minutes. Stain the nuclei with Mayer’s haematoxylin 5m.

Wash in water: 5 minutes. Dehydrate in 3 different jars of ufofab, clear in xylene and mount in a biological mountant or a drop of pure, natural honey.

Results:
Positive reactions are blue staining for acid mucopolysaccharides, magenta coloration for neutral mucopolysaccharides, and purple for a mixture of both.

2. ALCIAN BLUE AT PH 2.5 AND PH 1.0

Preparation of solutions
1% Alcian Blue 8gx in 3% acetic acid (pH 2.5).
0.5% Aqueous Neutral Red.

Methods
De-wax sections and take to water. Stain with Alcian blue solution for 5 minutes.
Wash in water, then counterstain with neutral red solution. Dehydrate in 3 changes of afobof, clear and mount in a bio-mountant or a drop of put honey.

Results:
Acid mucopolysaccharides stain blue, while nuclei stain red.

3. PERIODIC ACID-SCHIFF WITH ALCIAN BLUE COUNTERSTAIN

Preparation of solutions
1% Alcian Blue in 3% acetic acid.
1% aqueous periodic acid.
Schiff’s reagent (as used in method one)

Methods
De-wax sections and take to water. Stain with the Alcian blue solution for - 5 minutes.
Wash in tap water, then distilled water. Treat with the periodic acid solution for 2 minutes.

Wash in distilled water and treat with Schiff’s reagent for 8 minutes. Wash in running tap water for 10 minutes. Dehydrate, clear and mount in biomountant or pure honey.

Results:
Positive reactions are blue staining for acid mucopolysaccharides, magenta coloration for neutral mucopolysaccharides, and purple for a mixture of both.

4. AZUR A RE-AGENT

Preparation of solutions
1% aqueous potassium permanganate.
5% aqueous oxalic acid.
0.2% aqueous uranyl nitrate. Methods

Methods
De-wax sections and take to water. Treat with the potassium permanganate for 5 minutes.
Wash briefly and bleach in the oxalic acid solution. Wash wall in running tap water and stain with the Azur A solution for 5 minutes. Wash briefly in tap water and differentiate in the uranyl nitrate solution for 10-30 seconds, wash and blot dry. Dehydrate, clear and mount in bio-mountant or a drop of pure honey.

Results:
Stock saturated aqueous solution of Thionin, from which is prepared the working solution by adding 0.3ml of filtered stain to 50ml of tap water.

Methods:
De-wax sections and take down to water. Stain in the Thionin solution for 20 minutes. Wash in tap water and differentiate in the acetic acid solution for 2 minutes. Rinse in water, dehydrate, clear and mount in biomountant or pure honey.

Results:
Acid mucopolysaccharides stain purple to red, while neutral mucopolysaccharides stain blue.

6. HALE COLLOIDAL IRON

Preparation of solutions
Dialyzed iron and 2m acetic add.
2% aqueous potassium ferrocyanide.
2% aqueous hydrochloric acid.

Methods
De-wax and take sections to water. Treat test section with
USEFULNESS OF METACHROMATIC REAGENTS IN DISCRIMINATING BETWEEN CONNECTIVE TISSUE AND EPITHELIAL MUCOPOLYSACCHARIDES

Dialyzed iron solution for 10 minutes only. Wash well in distilled water. Dehydrate, clear and mount in biomountant or pure honey.

Results:
Acid mucopolysaccharides stain blue, while other structures demonstrate no coloration.

7. SOUTHGATE MUCICARMINE
Preparation of solutions
Carmine — 19g
Aluminium Hydroxide — 100ml
Mix and add 0.5g of aluminium chloride, boil in water bath for 14 minutes, cool and make up to original volume by adding 50% alcohol.

Methods
Bring section to water. Stain nuclei with Mayer’s haematoxylin. Blue for 5m in running tap water. Stain for 3am in the Southgate mucicarmine solution and rinse in distilled water. Dehydrate 3 changes of absolute alcohol, clear in xylene and mount in any biomountant or a drop of honey.

Results
Mucopolysaccharides stain red, while nuclei stain blue.

8. HEXAMINE SILVER
Preparation of solutions
Stock Hexamine silver solution: Take 5ml of 5% aqueous silver nitrate solution. Add 100ml of 3% aqueous Hexamine solution and mix. A white precipitate will form, which dissolves on further mixing. Store at 40°C in a dark bottle.

To use
Take 2ml of 5%, aqueous borate solution and add 25ml of distilled water mix and add 25ml of the stock Hexamine solution. 5% aqueous chromic acid. 1% aqueous solution of metabisulphite. 5% aqueous sodium thiosulphate (hypo) 0.2% light green in 0.2% acetic acid.

Methods
De-wax sections and take to water. Treat with the chromic acid solution for 1 hour. Wash and bleach with the sodium metabisulphite. Solution for approximately 1 minute. Wash well in tap water and then in distilled water. Place in a pre-heated Hexamine silver solution at 50°C for 15 minutes. Wash well in distilled water. Then wash and fix in 5% sodium thiosulphate (hypo) for 5 minutes. Wash, then counterstain in the light green solution for 4—1 minute. Wash, dehydrate, clear and mount in either natural biomountant or a drop of pure honey.

Results
Add mucopolysaccharides stain black, while the background is green.

9. BLOCKING OF REACTIVE METHYL GROUP
a. Methyl Esterification
Preparation of solution
0.1N (0.8%) Hydrochloric Acid in methanol.
1% Alcan Blue in 3% acetic acid.
0.5% aqueous neutral red.

Methods
De-wax sections and take down to water. Place test section and control in pre-heated reagent in the above solution for 4 hours at 37°C. Place, duplication sections of the above in distilled water for 4 hours at 37°C. These will constitute the negative controls. Wash all sections in water. Stain all sections by the standard P.12.5 Alcian blue stain, Counterstain with Neutral Red. Dehydrate in three changes of absolute India ink, clear in xylene and mount in bio-mountant or pure honey.

Results
Acid mucopolysaccharides with blue. Control and other inclusions will show no bluish colour.

b. Saponification
Preparation of solutions
0.1N (8%) Hydrochloric acid in methanol. 1% Potassium Hydroxide in 30% alcohol.
1% Alcian Blue in 3% acetic acid.
0.5% Aqueous Neutral red.

Methods
Prepare 2 sets of slides, one set consisting of 3 identical sections of the test material labelled A, B, and C. The other set should consist of 3 identical sections of a known positive material and again, labelled A, B, and C. De-wax and take sections to water. Please the two A and the two B sections in pre-heated reagent 1 above for 5 hours at 60°C and the two C sections in distilled water for the same period at 60°C. Wash all C sections well in water. Treat the two A sections with the saponification reagent for 30 minutes at room temperature and the two B and C sections with 70% alcohol for the same period of time. Wash all section well in water and stain in P.12.5 Alcian blue. Dehydrate in three changes of absolute alcohol first, then clear in xylene and mount in bio-montant or pure honey.

Results:
In the sections marked A any blue colour is an indication of positive reaction of add mucopolysaccharides only. The B sections show no Alcian blue colour at all, C shows no loss of Alcian Blue staining.
Phenyl Hydrazine and Pas Method

Preparation of solutions
1% aqueous periodic acid.
5% aqueous phenylhydrazine in Hydrochloride, Schiff’s reagent (see above).

Methods
De-wax and take sections to water. Treat all sections, test and
control with the periodic acid solution for 2 minutes. Wash well
in distilled water. Treat the test and positive control sections
with the phenylhydrazine solution for one hour at room
temperature and the negative control sections with distilled
water for the same period of time. Wash well in distilled water,
treat all sections with Schiff’s reagent for 8 minutes. Wash in
running tap water for approximately 10 minutes. Stain the nuclei
with Gill’s haematoxylin for 5 minutes and blue under running
tap water for 5 minutes. Dehydrate in three changes of absolute
palm wine gin-ufobob, clear in xylene and mount in bionmountant
or pure honey.

Results:
Acid mucopolysaccharides stain magenta, while neutral
mucopolysaccharides are negative.

40. ACRIDINE ORANGE
Preparation of solutions
4 aqueous ferric ammonium sulphate (Iron Alum).
0.1% aqueous Acridine orange.

Methods
De-wax and take sections to water. Treat with the iron alum
solution for 15 minutes. Wash well in water. Stain with the
Acridine orange solution for 11 minutes. Wash, plot dry and
mount in a drop of pure honey.

Results:
Acid mucopolysaccharides stain bright orange red, against a dull
green background.
Tissues stained using reagents 1 - 9 were examined using routine
light microscopy, whereas those stained with Acridine orange
were examined by fluorescence microscopy.

RESULTS

Staining reactions of normal tissues
Colonial mucosa, endocervical mucosa and glands,
salivary glands and duodenal Brenner’s glands demonstrated
variable positivity with most of the stains tested but were
consistently negative with Azur A and Thionin B (Table 1).
Bronchial cartilage and umbilical cord gave positive
reactions with both Azur A and Thionin B, as well as with all
other stains, except periodic acid Schiff in the case of umbilical
cord (Table 1).

Table 1:
Distribution of specific mucopolysaccharides in the baay

<table>
<thead>
<tr>
<th>MUCOPOLYSACCHARIDES</th>
<th>WHERE FOUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Chondroitin Sulphate A</td>
<td>Cartilage</td>
</tr>
<tr>
<td>2) Chondroitin Sulphate B</td>
<td>Heart valves, aorta, skin</td>
</tr>
<tr>
<td>3) Chondroitin Sulphate C</td>
<td>Cartilage, aorta, umbilical cord, skin</td>
</tr>
<tr>
<td>4) Hyaluronic sulphate</td>
<td>Cornea</td>
</tr>
<tr>
<td>5) Heparan sulphate</td>
<td>Mast cells, aorta</td>
</tr>
<tr>
<td>6) Keratan sulphate</td>
<td>Cartilage, nucleus pulposus</td>
</tr>
<tr>
<td>7) Hyaluronic acid</td>
<td>Synovium, skin, bone, cartilage, umbilical cord</td>
</tr>
</tbody>
</table>

Staining reactions of diseased tissues
Consistently positive staining reaction were obtained with
all of the stains tested in the epithelial cells and secretion of
colonial adenocarcinoma, as well as in the stroma of pleomorphic
salivary adenoma and chondrosarcoma (Table 2).
The Azur A stain was consistently negative in mucinous
cystadenoma of the ovary, mucinous salivary gland cyst,
mucinous carcinoma of the breast and invasive ductal carcinoma.
The Thionin stain was also negative in all of these lesions, apart
from one of the two invasive ductal carcinomas tested, which
gave faint, positive reaction (Table 2).

Specific staining methods
1. periodic acid-Schiff diastase stain
All the tissues tested gave positive reactions with periodic
acid-Schiff reagent after diastase digestion except for
normal colon and umbilical cord (Table 1 and 2).

2. Azur A and Thionin stains
The only tissue which gave consistently positive
reactions with the metachromatic Azur A and Thionin
B were the connective tissue stroma of umbilical cord,
cartilage (Figures 1 and 2), chondrosarcoma and
### TABLE 2: STAINING REACTIONS OF DISEASED TISSUES WITH DIFFERENT TECHNIQUES FOR DEMONSTRATING MUCOPOLYSACCHARIDES

<table>
<thead>
<tr>
<th>TYPES OF TISSUES</th>
<th>PAS/DIASTASE</th>
<th>AZUR A</th>
<th>THIONIN</th>
<th>ALCIAN BLUE (PH 2.5)</th>
<th>ALCIAN BLUE (PH 1.0)</th>
<th>SOUTH GATE</th>
<th>PAS/ALCIAN BLUE</th>
<th>HALE</th>
<th>METHENA MINE SILVER</th>
<th>ACRIDINE ORANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLON CARCINOMA</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+/+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>MUCINOUS CYSTADENOMA (OVARY)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+/+</td>
<td>+</td>
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<tr>
<td>PLEOMORPHIC SALIVARY ADENOMA</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>MUCINOUS SALIVARY CYST</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>MUCINOUS CARCINOMA (BREAST)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>INVASIVE DUCTAL BREAST CARCINOMA</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>CHONDROSARCOMA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>FIBROADENOMA (BREAST)</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>+/+</td>
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</tr>
</tbody>
</table>

pleomorphic salivary adenoma, as well as, the epithelial and secretions of adenocarcinoma of the colon (Table 1 and 2).

3. **Alcian blue Stain**
   At pH 2.5 all of the tissues tested apart from Brunner’s glands were alcianophilic. At pH 1.0 normal endocervix and respiratory glands gave a faint positive reaction, while mucinous cystadenoma of the ovary and mucinous carcinoma of the breast lost their alcianophilia (Tables 1 and 2).

4. **Southgate mucicarmine stain**
   The only tissues that were not mucicarminophilic were duodenal Brunner’s glands (Tables 1 and 2).

5. **Combined periodic acid-Schiff/Alcian blue stain**
   Periodic acid-Schiff appeared to have greater affinity for
epithelial, and Alcian blue for connective tissue mucosubstances in pleomorphic salivary adenoma and invasive ductal carcinoma of the breast.

6. Hale colloidal iron stain
AU the tissue mucopolysaccharides tested, except for those of normal duodenal Brunner's glands umbilical cord and salivary gland were positive with Hale colloidal iron (Tables 1 and 2).

7. Methenamine silver stain
The only tissue mucopolysaccharides tested which gave a negative reaction with methenamine silver stains were those of normal colon, endocervix and Brunner's glands (Table 2). The positive reaction obtained was a homogenous dark-brown colour, which was difficult to distinguish from background staining of other tissues, such as collagen and muscle.

8) Blocking of reactive methyl groups
a) Methyl esterification
Methyl esterification did not alter the alcianophilia of most normal and diseased tissues, except for adenocarcinoma of the colon, which gave a negative reaction after methyl esterification (Table 3).

b) Saponification
Most tissues apart from salivary gland, cartilage and mucinous cystadenoma of the ovary lost their alcianophilia after saponification (Table 3).

c) Phenylhydrazine treatment
All tissues, except normal colon, pleomorphic salivary adenoma and chondrosarcoma gave a positive periodic acid-Schiff reaction after phenylhydrazine treatment. Two of the mucinous breast carcinomas gave a positive reaction, while one was negative.

A major problem with the chemical blocking methods (methyl esterification, saponification and Phenylhydrazine treatment) was that for individual cases, several sections had to be prepared. This was because of both frequent lifting of sections after chemical treatment and difficulty in determining the end point of the reaction, resulting in loss of tissues.

9) Acridine orange Stain
All tissues tested, except for normal colon and cartilage, endocervix and Brunner's glands gave a positive fluorescence after Acridine orange treatment (Tables 1 and 2).

15 DISCUSSION
The results obtained in this study suggest that the most important discriminating stains between epithelial and connective tissue mucosubstances are the metachromatic dyes. In normal and diseased tissues, epithelial mucosubstances were generally orthochromatric, while connective tissue mucosubstances were metachromatric. The metachromatic dyes demonstrate acid mucopolysaccharides, both sulphated and non-sulphated (Culling, 1972). Neutral mucopolysaccharides are either weakly metachromatric or orthochromatric.

Despite the advice of Bancroft (1967) that Azur A washes off during the process of dehydration I observed that

<table>
<thead>
<tr>
<th>Tissues</th>
<th>10 percent formalin</th>
<th>70 percent</th>
<th>Industrial alcohol Ufob</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Colonic mucosa</td>
<td>****</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>2. Cartilage</td>
<td>****</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>3. Respiratory glands</td>
<td>****</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>4. Salivary gland</td>
<td>****</td>
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<td>***</td>
</tr>
<tr>
<td>5. Endocervix</td>
<td>****</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>6. Brunner's glands</td>
<td>****</td>
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<tr>
<td>7. Umbilical cord</td>
<td>****</td>
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<td>***</td>
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<tr>
<td>8. Skin</td>
<td>****</td>
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</tbody>
</table>

Table 3: Comparison of results of the preservation of mucosubstances following fixation in (a) 10 percent formalin (b) 70 percent industrial alcohol and (c) Raffia palm wine gin (ufob).

Two of the three mucinous breast carcinomas gave a negative Alcian blue reaction after saponification, while one was negative.
the value of the method lies in the fact that even after dehydration, metachromasia was still very well preserved. This was partly due to the use of uranyl nitrate solution for differentiation as well as pre-oxidation staining with

### Table 4: Staining reactions of normal tissues with different techniques for demonstrating mucopolysaccharides. Staining techniques

<table>
<thead>
<tr>
<th>Types of Tissue</th>
<th>PAS/Diastase</th>
<th>Alcian A</th>
<th>Thionin</th>
<th>Alcian Blue (PH 2.5)</th>
<th>Alcian Blue (PH 1.0)</th>
<th>South gate</th>
<th>PAS/Alcian blue</th>
<th>Hale</th>
<th>Methenamine Silver</th>
<th>Acidine Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td></td>
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<td>+</td>
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<tr>
<td>Endocervix</td>
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<td>+</td>
<td>±</td>
<td>+/+</td>
<td>+</td>
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<tr>
<td>Respiratory</td>
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<td>+</td>
<td>±</td>
<td>+/+</td>
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<td>glands</td>
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<td>Brunners glands</td>
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<tr>
<td>Cartilage</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Umbilical Cord</td>
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<td>+</td>
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</table>

### Table 5: Staining reactions of diseased tissues with different techniques for demonstrating mucopolysaccharides. Staining techniques

<table>
<thead>
<tr>
<th>Types of Tissue</th>
<th>PAS/Diastase</th>
<th>Alcian A</th>
<th>Thionin</th>
<th>Alcian Blue (PH 2.5)</th>
<th>Alcian Blue (PH 1.0)</th>
<th>South gate</th>
<th>PAS/Alcian blue</th>
<th>Hale</th>
<th>Methenamine Silver</th>
<th>Acidine Orange</th>
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<tbody>
<tr>
<td>Adenocarcinoma</td>
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<td>+</td>
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<td>+/+</td>
<td>+</td>
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<td>of Colon</td>
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<td>+</td>
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<tr>
<td>Mucinous</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cystadenoma</td>
<td></td>
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<td>+</td>
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<td>(ovary)</td>
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Table 6: Effects of blocking of reactive acid groups by methyl esterification and treatment with Phenylhydrazine in normal and diseased tissues.

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<th>Types of Tissues</th>
<th>METHYL ESTERIFICATION</th>
<th>SAPONIFICATION</th>
<th>PHENYLHYDRAZINE</th>
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<td>Fibroadenoma</td>
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Alcian blue does not stain other acidic substances of high density such as nuclear deoxyribonucleic acid and cytoplasmic ribonucleic acid, which is an advantage of this stain over Hale colloidal iron (Casselman, 1962).

The periodic acid—Schiff reaction after diastase treatment was a non-specific general purpose stain, useful in demonstrating but not in discriminating between epithelial and connective tissue mucopolysaccharides. This is not surprising since the positive reaction of mucosubstances with periodic acid-Schiff reagent is probably solely due to the presence of a hexose component and acidic groups such as hexuronic acid do not contribute significantly to the reaction.

The present study shows that Southgate mucicarmine and Hale colloidal iron stains are other good general-purpose stains for mucopolysaccharides. On the other hand, methenamine silver, Acidine orange, and blockage of reactive methyl groups were found technically inferior to Southgate's mucicarmine, Hale colloidal iron and metachromatic dyes, such as Azur A and Thionin B.

Therefore, it is recommended that metachromatic dyes such as Azur A and Thionin B are useful for routine demonstration, classification and discrimination of normal, and diseased tissue mucosubstances.

REFERENCES


USEFULNESS OF METACHROMATIC REAGENTS IN DISCRIMINATING BETWEEN CONNECTIVE TISSUE AND EPITHELIAL MUCOPOLYSACCHARIDES


