

# 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, Acridine 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 epithella 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 histological 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 (ufob) (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 1g of basic fuchsin to 200ml of boiling distilled water and dissolve. Cool to 50°C and 2g of Potassium metabisulphite. Dissolve, allow cooling to room temperature and add 2mls of concentrated Hydrochloric acid. Leave overnight in the dark, and then add 0.12g 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 acid 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 ufofob, 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 PH2.5 AND PH1.0

### Preparation of solutions

1% Alcian Blue 8gx in 3% acetic acid (pH2.5).

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 ufofob, clear and mount in a bio-mountant or a drop of pur 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 well 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.

0.2% glacial acetic acid.

### 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

2 minutes. Rinse in water, dehydrate, clear and mount in bio-mountant 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

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 24 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 xofob, 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 400c in a dark bottle.

**To use**

Take 2ml of a 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 56°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% Aldan Blue in 3% acetic acid.

0.5% aqueous neutral red.

**Methods**

De-wax section and take down to water. Place test section and control in pre-heated reagent in the above solution for 4 hours at 37oc. Place, duplication sections of the above in distilled water for 4 hours at 37oc. These will constitute the negative controls. Wash all sections in water. Stain all sections by the standard P.H2.5 Alcian Blue stain. Counterstain with Neutral Red. Dehydrate in three changes of absolute palm wine gm-xofob, 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 70% 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 600c 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 re-agent 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.H2.5 Alcian Blue. Dehydrate in three changes of absolute palm wine gm-xofob clear in cylene and mount in bio-mountant 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.

### c. 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-ufob, clear in xylene and mount in biomountant or pure honey.

#### **Results:**

Acid mucopolysaccharides stain magenta, while neutral mucopolysaccharides are negative.

### 10. 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**

Colonic 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**

| MUCOPOLYSACCHARIDES        | WHERE FOUND                                     |
|----------------------------|---|
| (1) Chondroitin Sulphate A | Cartilage                                       |
| (2) Chondroitin Sulphate B | Heart valves, aorta, skin                       |
| (3) Chondroitin Sulphate C | Cartilage, aorta, umbilical cord, skin          |
| (4) Hyaluronic sulphate    | Cornea  |
| (5) Heparan sulphate       | Maat cells, aorta                               |
| (6) Keratan sulphate       | Cartilage, nucleus pulposus                     |
| (7) Hyaluronic acid        | Synovium, skin, bone, cartilage, umbilical cord |

### **Staining reactions of diseased tissues**

Consistently positive staining reaction were obtained with all of the stains tested in the epithelial cells and secretion of colonic 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**

| TYPES OF TISSUES                 | PAS/DIAS TASE | AZUR A | THIONIN | ALCIAN BLUE (PH 2.5) | ALCIAN BLUE (PH 1.0) | SOUTH GATE | PAS/ALCIAN BLUE | HALE | METHENA MINE SILVER | ACRIDINE ORANGE |
|----------------------------------|---------------|--------|---------|----------------------|----------------------|------------|-----------------|------|---------------------|-----------------|
| COLON CARCINOMA                  | +             | +      | +       | +                    | +                    | +          | +/+             | +    | +                   | +               |
| MUCINOUS CYSTADENOMA (OVARY)     | +             | -      | -       | +                    | -                    | +          | +/+             | +    | +                   | +               |
| PLEOMORPHIC SALIVARY ADENOMA     | +             | +      | +       | +                    | +                    | +          | +/+             | +    | +                   | +               |
| MUCINOUS SALIVARY CYST           | +             | -      | -       | +                    | +                    | +          | +/+             | +    | +                   | +               |
| MUCINOUS CARCINOMA (BREAST)      | +             | -      | -       | ±                    | -                    | +          | +/+             | +    | +                   | +               |
| INVASIVE DUCTAL BREAST CARCINOMA | +             | -      | ±       | +                    | +                    | +          | +/+             | +    | +                   | +               |
| CHONDROSARCOMA                   | +             | +      | +       | +                    | +                    | +          | +/+             | +    | +                   | +               |
| FIBROADENOMA (BREAST)            | +             | -      | +       | +                    | +                    | +          | +/+             | +    | +                   | +               |

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

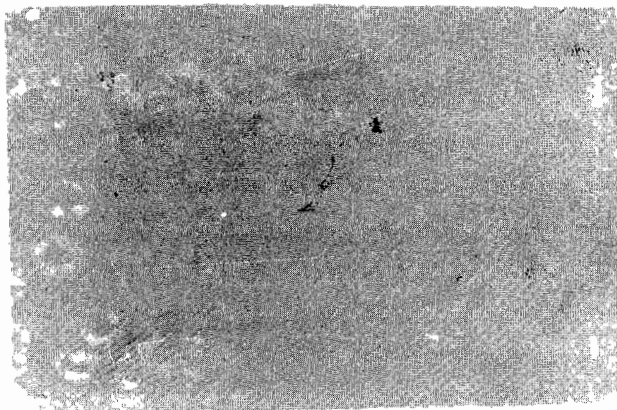


FIGURE I  
CHONDROSARCOMA SHOWING METACHROMATIC (REDDISH PURPLE) COLOUR IN THE CONNECTIVE TISSUE MATRIX WITH THIONIN. B REACTION ARROWED

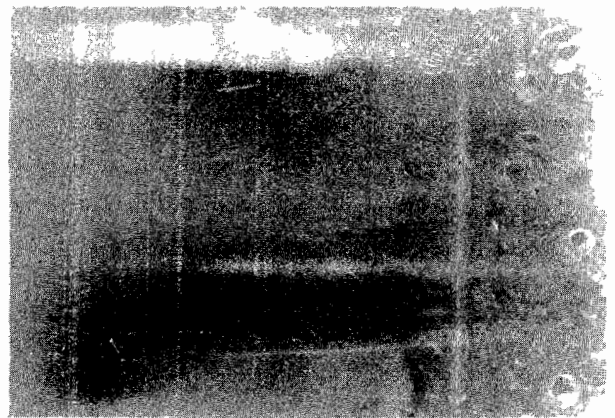


FIGURE II  
CHONDROSARCOMA DISPLAYING STRONGLY METACHROMATIC PURPLE COLOUR WITH GREENISH INTERSPACING BACKGROUND WITH AZUR A REACTION -- ARROWED  
AZUR A X 250

ovary and mucinous carcinoma of the breast lost their alcianophilia (Tables 1 and 2).

4. *Southgate mucicarmin stain*

The only tissues that were not mucicarminophilic were duodenal Brunner's glands (Tables 1 and 2).

5. *Combined periodic add-Schiff/Alcian blue stain*  
Periodic acid-Schiff anneared 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 1 and 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

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).

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## 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 orthochromatic, while connective tissue mucosubstances were metachromatic. The metachromatic dyes demonstrate acid mucopolysaccharides, both sulphated and non-sulphated (Culling, 1972). Neutral mucopolysaccharides are either weakly metachromatic or orthochromatic.

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

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 (ufofob).

| Tissues               | FIXATIVES           |            |                           |
|-----------------------|---------------------|------------|---------------------------|
|                       | 10 percent formalin | 70 percent | Industrial alcohol Ufobob |
| 1. Colonic mucosa     | ****                | *          | ***                       |
| 2. Cartilage          | ****                | *          | ***                       |
| 3. Respiratory glands | ****                | *          | ***                       |
| 4. Salivary gland     | ****                | *          | ***                       |
| 5. Endocervix         | ****                | *          | ***                       |
| 6. Brunner's glands   | ****                | *          | ***                       |
| 7. Umbilical cord     | ****                | *          | ***                       |
| 8. Skin               | ****                | *          | ***                       |

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

| Types of Tissues   | PAS/Diastase | Azur A | Thionin | Alcian Blue (PH.2.5) | Alcian Blue (PH.1.0) | South gate | PAS/Alcian blue | Hale | Methenamine Silver | Acridine Orange |
|--------------------|--------------|--------|---------|----------------------|----------------------|------------|-----------------|------|--------------------|-----------------|
| Colon              | --           | --     | --      | +                    | +                    | +          | --/+            | +    | --                 | --              |
| Endocervix         | +            | --     | --      | +                    | ±                    | +          | +/+             | +    | --                 | +               |
| Respiratory glands | +            | --     | --      | +                    | ±                    | +          | +/+             | +    | +                  | +               |
| Brunners glands    | +            | --     | --      | --                   | --                   | --         | +/-             | --   | --                 | +               |
| Cartilage          | +            | +      | +       | +                    | +                    | +          | +/+             | +    | +                  | --              |
| Umbilical Cord     | --           | +      | +       | +                    | +                    | +          | --/+            | --   | +                  | +               |

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

| Types of Tissues                 | PAS/Diastase | Azur A | Thionin | Alcian Blue (PH.2.5) | Alcian Blue (PH.1.0) | South gate | PAS/Alcian blue | Hale | Methenamine Silver | Acridine Orange |
|----------------------------------|--------------|--------|---------|----------------------|----------------------|------------|-----------------|------|--------------------|-----------------|
| Adenocarcinoma of Colon          | +            | +      | +       | +                    | +                    | +          | +/+             | +    | +                  | +               |
| Mucinous Cystadenoma (ovary)     | +            | --     | --      | +                    | --                   | +          | +/+             | +    | +                  | +               |
| Pleomorphic Salivary adenoma     | +            | +      | +       | +                    | +                    | +          | +/+             | +    | +                  | +               |
| Mucinous Salivary Cyst           | +            | --     | --      | +                    | +                    | +          | +/+             | +    | +                  | +               |
| Mucinous Carcinoma (breast)      | +            | --     | --      | ±                    | --                   | +          | +/+             | +    | +                  | +               |
| Invasive ductal breast Carcinoma | +            | --     | ±       | +                    | +                    | +          | +/+             | +    | +                  | +               |
| Chondrosarcoma                   | +            | +      | +       | +                    | +                    | +          | +/+             | +    | +                  | +               |
| Fibroadenoma (breast)            | +            | --     | +       | +                    | +                    | +          | +/+             | +    | +                  | +               |

Table 6: Effects of blocking of reactive acid groups by methyl esterification and treatment with Phenylthiazine in normal and diseased tissues.

| Types of Tissues          | METHYL ESTERIFICATION |             | SAPONIFICATION |             |             | PHENLHYDRAZINE |
|---------------------------|-----------------------|-------------|----------------|-------------|-------------|----------------|
|                           | A (TEST)              | B (Control) | A (TEST)       | B (Control) | C (Control) |                |
| Normal Colon              | +                     | +           | --             | +           | +           | --             |
| Endocervix                | +                     | +           | --             | +           | +           | +              |
| Respiratory glands        | +                     | +           | --             | +           | +           | +              |
| Salivary gland            | +                     | +           | +              | +           | +           | +              |
| Cartilage                 | +                     | +           | +              | +           | +           | +              |
| Colon adenocarcinoma      | --                    | +           | --             | --          | +           | +              |
| Mucinous Cystadenoma      | +                     | +           | --             | +           | +           | --             |
| Mucinous salivary cyst    | +                     | +           | +              | +           | +           | +              |
| Mucinous breast carcinoma | +                     | +           | --             | +           | +           | +              |
| Invasive ductal carcinoma | +                     | +           | --             | +           | +           | +              |
| Chondrosarcoma            | +                     | +           | --             | +           | +           | +              |
| Fibroadenoma              | +                     | +           | --             | +           | +           | +              |

potassium permanganate, both of which enhance metachromasia.

Strongly acidic mucopolysaccharides such as in the stroma or normal and diseased tissues, retained their alcianophilia at pH1. On the other hand mucopolysaccharides such as in endocervix, respiratory glands and mucinous cystadenoma of the ovary gave a negative reaction at pH1, but reacted positively at pH2.5.

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, Acridine 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.

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