

## Alcian Blue 8GS: A New Stain for Mucin

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### SUMMARY

A new method of staining mucin is described. The stain used is alcian blue 8GS. The method is rapid and easy in application. The results are clear and permanent.

THE disadvantages of most staining methods employed for the demonstration of mucin in histological sections of animal tissues are as follows:

1. Doubtful specificity. Not only mucins accept the stain; other elements such as nuclei, connective tissues, &c., may do so as well.
2. Loss of stain. In some cases where mucin is stained in a specific manner by dilution of the stain used, by adjustment of the pH of the stain solution, or by metachromatic properties of the stain, it is frequently difficult or impossible to dehydrate, clear, and mount the section without either immediate or later loss of the stain.
3. The stained mucin may have other stains added to it during additional operations.
4. The staining methods may be involved or may require the making-up of reagents which are often unstable.

'Alcian' blue 8GS has only one of these defects—it will not distinguish chondroitin sulphuric acid complexes from mucoitin sulphuric acid complexes. It therefore stains cartilage and mucin equally. Its advantages are that it is easy to apply; that it stains mucin clearly and conspicuously; that it combines with mucin in such manner that additional stains produce little or no alteration in colour. It reacts with mucin with such vigour that all common histological reagents fail to dislodge it, and only prolonged treatment with acid alcohol (2–24 hours) will reduce an overstained section to the required intensity. Once applied it will resist indefinitely water, alcohols, alkalis and hydrocarbons, and weak acid solutions for short periods of time. Preparations stained in 1947 and mounted in balsam, 'sira', and 'distrene' show no sign of fading.

Alcian blue 8GS is produced by Imperial Chemical Industries Ltd., to whom thanks are due for supplying dyes from time to time. It is a water-soluble precursor of the pigment monastral fast blue, into which it may be turned by action with alkalis. Industrially it is used in the dyeing of cotton.

It is not suggested here that alcian blue is a specific stain for mucin. Indeed, if staining is prolonged it will stain every tissue in a section except the nucleus.

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It is claimed, however, that if mucin is present alcian blue 8GS can be so used that only the mucin will be stained.

Tissues fixed in picric acid or mercuric chloride fixatives (Bouin, Susa, Zenker, &c.) give the best results. Formaldehyde material is not good.

#### METHOD

1. Bring paraffin wax or ester wax (Steedman, 1947) sections down to water in the usual way.
2. Stain with filtered 1 per cent. alcian blue in distilled water for 10-40 seconds. If longer times are given other tissues will take up the stain.
3. Rinse in distilled water to remove the excess stain solution.
4. Stain with haemalum 5-10 minutes.
5. Continue with the normal haemalum technique, counterstaining with eosin or whatever other additional stains may be required.
6. Dehydrate, clear in xylene, mount in balsam or any other resinous medium.

This method will stain any mucin a clear blue-green colour and the addition of the other two stains will in no way affect it. Where it is feared that an aqueous staining solution may dissolve imperfectly-fixed mucin, the ribbon staining method with ester wax sections may be employed.

Generally it takes so long using acid alcohol to remove excess alcian blue from a section that it is better to use the stain progressively as indicated, and the maximum time of 40 seconds is rarely exceeded with advantage.

Dilution of the stain to 0.1 per cent. or even to 0.01 per cent. does not appear to confer any particular advantage on the staining procedure.

The method has so far been used on a variety of vertebrate and invertebrate material without failure.

Should the use of stains in acid solution be contemplated after using alcian blue, two courses may be followed to counteract the slight destaining action of such fluids:

1. The section may be slightly overstained with alcian blue. This would mean 40-60 seconds in a 1 per cent. aqueous solution.
2. The section could be stained for the usual 10-40 seconds, rinsed in water, and taken up to alkaline 70 per cent. alcohol (pH 8 or over). Left in this solution for 2 hours or longer the bonds which make alcian blue a soluble dye are broken down and it becomes the insoluble pigment monastral fast blue, which will resist any further histological reagents. The longer the stained section remains in alkaline alcohol the more insoluble the stain becomes.

These modifications are not required when using dye solutions which are weakly acid, neutral, or alkaline.

It should be noted that while such dyes as thionin, methylene blue, toluidin blue, &c., give polychromatic effects in sections which contain mucin, macrophages, and tissue basiphils, alcian blue does not. It stains only mucin, and macrophages and tissue basiphils remain unstained. (Jorpes, Holmgren, and Wilander (1937) believe that the staining constituent of tissue basiphils is heparin.)

In aqueous solution alcian blue develops mould very rapidly. Thymol may be used to reduce the rate of growth, but even then filtration is advisable every 10 days. A freshly made solution stains best.

Alcian blue 8GS is a phthalocyanin dye and its chemical constitution is discussed by Haddock (1948).

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