A histochemical study of simple laryngeal polyps

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SUMMARY Benign laryngeal polyps and normal larynxes have been studied by histochemical and other staining methods. These show that the polyps contain much free hyaluronic acid and 6-chondroitin sulphate. Their mode of production may be the mechanical separation of these compounds from collagen fibres by vibration. An alcian-elastic stain may be a helpful diagnostic method.

Laryngeal polyps (singer's nodes) are familiar to laryngologists and surgical histopathologists, but their pathogenesis has not been agreed. The histological features of laryngeal polyps have similarities with those of myxoedema of the larynx, and this study sets out to determine whether there are histochemical changes in laryngeal polyps and myxoedema of the larynx that could be diagnostically useful or throw light upon their pathogenesis.

Ogawa has demonstrated alcianophilia in laryngeal polyps that was abolished by pretreatment with hyaluronidase and chondroitinase, but Zbořil and Nožička were unable to show differences between the normal larynx and laryngeal polyps by the periodic acid-Schiff (PAS) technique. The ground-substance of the myxoedematous larynx has been found to contain acid mucopolysaccharides which were also present in the true vocal cord of the hypothyroid rat.

It has been shown that different types of acid mucopolysaccharides show alcianophilia at varying pH or at critical electrolyte concentrations and compete in characteristic ways with other dyes. These techniques have been used to compare laryngeal polyps and the myxoedematous larynx with the normal vocal cord.

Material and methods

Twenty-five laryngeal polyps were examined. Control material from 16 larynxes showing no morphological abnormality was obtained by taking transverse blocks across the vocal cords in a similar manner to that of Browning et al. A biopsy from a patient with myxoedema of autoimmune aetiology who presented with hoarseness of voice was also available.

All the polyps had been diagnosed independently as showing characteristic histological features of the lesion, including vascular proliferation, fibrosis, or a myxoid appearance of the underlying stroma.

The specimens were all fixed for 24 hours in Lillie's buffered formalin, post-fixed for 16 hours in formol-corrosive, dehydrated in alcohol, cleared in toluene, and embedded in paraffin wax. Sections were cut at 6 µm. Sections were stained by the following methods: alcian blue at pH 0-2, 1-0, and 2-5; alcin blue at pH 2-5 after treatment with hyaluronidase; alcian blue/aldehyde fuchsin and alcian blue/PAS at pH 2-5, and Weigert's elastic stain. Additionally, some sections were stained with Congo red for amyloid and by the martius scarlet blue (MSB) technique for fibrin.

Results (see table)

The normal laryngeal connective tissue gave negative or incomplete weak staining with alcian blue at pH 0-2 but very little alcianophilia at pH 1-0 and 2-5. A band of elastic tissue parallel to the surface

<table>
<thead>
<tr>
<th>Staining method</th>
<th>Normal tissue 16 studied</th>
<th>Laryngeal polyps 25 studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of alcianophilia</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Alcian blue, pH 0-2</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Alcian blue, pH 1-0</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Alcian blue, pH 2-5</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Alcian blue/PAS</td>
<td>16 PAS + ve</td>
<td>PAS – ve 25</td>
</tr>
<tr>
<td>Alcian blue/aldehyde fuchsin</td>
<td>16 fuchsin + ve</td>
<td>Variable results</td>
</tr>
<tr>
<td>Weigert's elastic</td>
<td>16 positive</td>
<td>Negative 25</td>
</tr>
</tbody>
</table>

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epithelium was fuchsinophil with alcian blue/ 
aldehyde fuchsin and PAS positive with alcian 
blue/PAS.

By contrast, the stroma of the singer's nodes 
showed strong and consistent alcianophilia at 
\( \text{pH 0-2 and 2-5 with positive but less strong staining} \) 
at \( \text{pH 1-0; all these reactions were abolished by} \) 
pretreatment with hyaluronidase. Elastica was 
virtually absent when stained by the Weigert 
technique, and there was partial loss of fuchsinophila 
with alcian blue/aldehyde fuchsin. The 
myxoedema case showed the presence of elastic 
tissue but the stromal connective tissue stained as a 
singer's node. No fibrin or amyloid was demon-
strated by the MSB or Congo red methods.

Discussion

Acid mucopolysaccharides combine with alcian 
blue at different pH levels according to their 
composition. At low pH sulphated radicals become 
ionised and react, while unsulphated radicals do not. 
At pH 2-5 most acid mucopolysaccharides will 
stain with alcian blue. Those radicals which are 
finely attached to collagen will not react under any 
conditions, and large molecules present fewer 
binding sites for a given molecular weight than 
small ones. The staining reactions of the collagen of 
the normal larynx suggest that there is a little 
sulphated material but no unsulphated mucopoly-
saccharides. In the singer's nodes the mucopoly-
saccharides appear to be of smaller molecular size, 
to be less avidly linked to collagen, and to consist of 
highly sulphated and unsulphated compounds. These 
findings would be consistent with the presence of 
chondroitin sulphates and hyaluronic acid in the 
singer's nodes while the normal larynx contains a 
little 6-chondroitin sulphate but is free from 
hyaluronic acid, confirming Ogawa's work using 
less specific chondroitinases. In the myxoedematous 
larynx alcianophilia at varying pH has been demon-
strated, and although Bicknell\(^3\) thought that this was 
diagnostic, the present study has shown that similar 
staining reactions are found in singer's nodes, and 
an elastic stain would be helpful in distinguishing 
these changes.

In incomplete ultrastructural studies Busuttill\(^10\) 
has suggested that the mechanical effects of vibration 
broke off small fragments from collagen fibres in 
singer's nodes. It is postulated that the presence of 
hyaluronic acid and 6-chondroitin sulphate is due 
to these compounds being freed from collagen by 
high-frequency vibration.

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