The diagnostic value of mucosubstances in rectal biopsies from patients with ulcerative colitis and Crohn’s disease

M. I. FILIPE AND IAN DAWSON
From the Department of Clinical Histochemistry, Westminster Medical School, London

SUMMARY Sixty-two biopsies from 36 patients with ulcerative colitis and 29 biopsies from 15 patients with Crohn’s disease were studied with special reference to the content of neutral, acid non-sulphated and sulphated mucosubstances. A marked decrease was found in mucosubstance in patients with active ulcerative colitis and was related to the intensity of inflammation in the lamina propria but not to the duration of the disease. In patients with active Crohn’s disease there was no corresponding decrease. Techniques for mucosubstances appear to be of value in differentiating between the two diseases in rectal biopsies.

The importance of rectal biopsy in the diagnosis and follow up of ulcerative colitis and Crohn’s disease of the large intestine has been emphasized by Matts (1961), Gonzalez-Licea and Yardley (1966), and others. Biopsy also has an important role in attempts to separate these two diseases. There are, however, limitations to the value of conventional histology; for example the histological lesions found in ulcerative colitis are not always specific and can be inseparable from those seen in other inflammations such as bacillary dysentery. Nor is histology always helpful in the differential diagnosis between ulcerative colitis and Crohn’s disease when pathological features of both are present (Lewin and Swales, 1966).

For these reasons many workers have turned to histochemical or ultrastructural techniques for the further evaluation of such biopsies. Ultrastructural changes have been described as of value in detecting disease activity (Gonzalez-Licea and Yardley, 1966) but none have so far been described which are diagnostic of ulcerative colitis or of Crohn’s disease. Monis and Mendeloff (1965) described characteristic changes in the distribution of NADP-linked dehydrogenases and non-specific esterases in ulcerative colitis, but these findings have not been confirmed by other workers (Van Noorden, Thayer, Yesner, and Spiro, 1967; Melnyk, Braucher, and Kirsner, 1967; Filipe, 1968), and there are no ultrastructural changes at the epithelial cell apex to correlate with them.

Other workers have turned to a study of mucosal mucins in colectomy specimens from ulcerative colitis (Greco, Lauro, Fabbrini, and Torsoli, 1967) and ulcerative colitis and Crohn’s disease (Hellstrom and Fisher, 1967). This seems a profitable line of approach, bearing in mind that rectal biopsy is easy and relatively innocuous and is being increasingly used by clinicians as a complement to clinical, radiological, and sigmoidoscopic investigations. It was decided to review all rectal biopsies of confirmed cases of ulcerative colitis and Crohn’s disease of the large bowel seen in the Vincent Square Laboratories of Westminster Hospital over the past ten years, using histochemical techniques for mucosubstances to assess their value as an aid to definitive differential diagnosis between the two conditions.

Material and Methods
The studies presented here were on rectal biopsies from patients treated at the Gordon Hospital (Westminster Group) during the period 1958 to 1968 and in all of them a diagnosis of either ulcerative colitis or Crohn’s disease of the large
HISTOCHEMICAL FINDINGS

The age at first onset of symptoms lay between 13 and 78 years (mean 45.5) for 36 patients with ulcerative colitis, with a fairly even scatter between the third, fourth, and fifth decades and a considerable number beginning in the seventh decade, and between 11 and 50 years (mean 30.5) for 15 cases with Crohn’s disease with a maximum incidence in the second and third decades. The duration of symptoms up to the time of biopsy varied from one month to 23 years in colities, being less than four years in the majority, and in Crohn’s disease it varied from four to 27 years, being four to eight years in the majority. The sex incidence was equal in both ulcerative colitis and Crohn’s disease.

Table I Histological classification of the cases studied

<table>
<thead>
<tr>
<th>Disease</th>
<th>Histology</th>
<th>Acute</th>
<th>Chronic</th>
<th>Quiescent</th>
<th>Fulminant</th>
<th>Non-specific</th>
<th>Normal</th>
<th>Typical (Group I)</th>
<th>Borderline (Groups 2-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerative colitis (36 patients)</td>
<td></td>
<td>11</td>
<td>32</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 62 rectal biopsies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease (15 patients)</td>
<td></td>
<td>5</td>
<td>4</td>
<td>15</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 29 rectal biopsies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to classification of Lewin and Swales (1966)

The classification of mucosubstances is confused (Spicer, Leppi, and Stoward, 1965) and it is not proposed to discuss it here. Epithelial mucosubstances are probably all glycopoly-

peptides in which carbohydrates are linked to the side chains of amino acids forming the poly-peptide strand through covalent bonds (Jeanloz, 1960, 1963; Gottschalk, 1962). The carbohydrate units are either disaccharides or polysaccharides of low molecular weight, often with a terminal sialic acid group. Histocnemical techniques identify the carbohydrate as opposed to the poly-peptide component, and allow a simple classification into neutral, acid non-sulphated, and sulphated mucosubstances. The following techniques have been used on sections from the original paraffin blocks. Periodic acid-Schiff to test for neutral mucosubstances, alcin blue at pH 2.5 followed by periodic acid-Schiff to test for acid non-sulphated and neutral mucosubstances (Mowry and Morard, 1957), and high iron diamine followed by alcin blue at pH 2.5 for acid sulphated and acid non-sulphated mucosubstances (Spicer, 1965).

The amount of mucosubstance present was estimated semiquantitatively on a visual basis using a scale from 0 to ++++ with ++++ representing the normal.

Results

CLINICAL FINDINGS

The age at first onset of symptoms lay between 13 and 78 years (mean 45.5) for 36 patients with ulcerative colitis, with a fairly even scatter between the third, fourth, and fifth decades and a considerable number beginning in the seventh decade, and between 11 and 50 years (mean 30.5) for 15 cases with Crohn’s disease with a maximum incidence in the second and third decades. The duration of symptoms up to the time of biopsy varied from one month to 23 years in colities, being less than four years in the majority, and in Crohn’s disease it varied from four to 27 years, being four to eight years in the majority. The sex incidence was equal in both ulcerative colitis and Crohn’s disease.

FIG. 1 Normal rectal mucosa to show sulphated mucosubstance (dark staining high iron diamine and alcian blue) predominantly in the lower two-thirds and a mixture of sulphated and acid non-sulphated mucosubstance in the upper third. ×60
The diagnostic value of mucosubstances in rectal biopsies

Table II Variations in the amount of mucosubstance in mucosa in relation to the histological picture

<table>
<thead>
<tr>
<th>Histology</th>
<th>o/+</th>
<th>++/++/+</th>
<th>++/++++</th>
<th>Variable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerative Colitis</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>18</td>
<td>9</td>
<td>2</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Chronic active</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Quiescent</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulminant</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-specific lesions</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>26</td>
<td>7</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Table III Relationship between the amount of mucus in the mucosa and the degree of inflammation in lamina propria

<table>
<thead>
<tr>
<th>Amount of Mucus</th>
<th>Ulcerative Colitis</th>
<th>Crohn's Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>o/+</td>
<td>++/++/+</td>
</tr>
<tr>
<td>Inflammatory Infiltrate</td>
<td>or Variable</td>
<td>or Variable</td>
</tr>
<tr>
<td>Heavy</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Mild or normal</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>17</td>
</tr>
</tbody>
</table>

contain a mixture of sulphated and non-sulphated acid mucosubstances, with a variable small amount of neutral mucosubstance (Figure 1). The rectal mucosa of children aged from 2 days to 8 years shows a similar pattern (Filipe, 1969).

An attempt was made to classify any alteration in the amount of mucosubstance in the biopsies in terms of sulphated, acid non-sulphated, or neutral mucosubstance. In any general reduction in the amount of mucosubstance, both sulphated and acid non-sulphated mucosubstances decrease, but this reduction affects the non-sulphated less than the sulphated. No increases above normal were found in any mucosubstances.

Table II shows the semiquantitative findings for mucosubstances related to the different histological patterns in ulcerative colitis and in Crohn’s disease. In both diseases where polypoid zones were present in the biopsies non-polypoid zones were chosen without extensive ulceration for the estimations, since it was found that polypoid zones invariably contained abundant mucosubstances, and severely ulcerated zones have insufficient mucosa for reliable estimates. The findings show clearly a decrease in mucosubstances in the majority of cases of ulcerative colitis but no corresponding decrease in Crohn’s disease (Figures 2 to 6).

A correlation of the amount of mucosubstance present with the duration of disease was next attempted but no positive correlation could be found. An attempt to correlate the amount of mucosubstance with the degree of inflammation in the lamina propria was (Table III) more successful. In the colitic group, 36 of the 62 rectal biopsies had a heavy inflammatory cellular infiltrate in the lamina propria (Fig. 2), and 29 in this group had a marked generalized decrease in mucosubstance and four a patchy decrease (Fig. 3), while only three had normal amounts. Eighteen biopsies showed a moderate inflammatory infiltrate of which 11 showed a marked decrease in mucosubstances, while seven showed slight decreases. Eight showed mild inflammatory

Fig. 2 Rectal mucosa in chronic active ulcerative colitis. (Haematoxylin and eosin) ×52.

Fig. 3 Serial section of biopsy in Fig. 2 to show the marked decrease in mucosubstance. (High iron diamine and alcian blue) ×51.
infiltrate, and three of these showed moderate decrease in mucosubstances. In the group with Crohn’s disease, although eight biopsies showed severe and 21 moderate or mild inflammatory changes in the lamina propria (Figs 5 and 6), the mucosubstances were reduced only in two of the biopsies.

Finally those patients were considered from whom two or more biopsies were available at intervals and an attempt made to was correlate the amount of mucosubstance present with the progression, or regression, of the disease as judged histologically. It was found that, in general, healing and mucosal regeneration with diminution of inflammatory reaction, parallel an increase in mucosubstances (Figures 7 and 8).

It will be clear from these findings that most cases of ulcerative colitis show a decrease in mucosubstance while most cases with Crohn’s disease do not. Fourteen out of the 91 biopsies, all diagnosed as ulcerative colitis, showed a normal mucosubstance content which on this supposition was not in keeping with the histological diagnosis. Before regarding these as a definite negative correlation it was decided to review both the original biopsies and the operation specimens, since in the laboratory the separation of large intestinal Crohn’s disease from ulcerative colitis was only made with any confidence after 1963.

Of these 14 cases, three were reclassified as Crohn’s disease, three confirmed as ulcerative colitis while eight still defied positive identification, falling into types II and III of Lewin and Swales (1966) in which there are macroscopic and microscopic features suggestive, but not conclusive, of Crohn’s disease. The experience in a considerable number of such cases points to conclusion that where, after considering the clinical history, radiology, and histology of a resected colon, there is still doubt as to whether the condition is Crohn’s disease or colitis, the disease will behave as Crohn’s disease rather than colitis. If this be accepted, then the normal mucosubstance is in keeping with this opinion.

Discussion

When a patient presents with clinical evidence of proctitis or colitis, and specific bacterial or protozoal inflammation can be excluded, the diagnosis usually rests between non-specific proctitis, ulcerative colitis, and Crohn’s disease, and then rectal biopsy is often performed to elucidate it. Although there are specific histological features which may allow of a definite diagnosis
of Crohn's disease or of ulcerative colitis (Lockhart-Mummery and Morson, 1960; Janowitz and Present, 1966; Hawk and Turnbull, 1966; Gonzalez-Licea and Yardley, 1966) two problems commonly arise. First the biopsy may show only 'non-specific inflammation' and the diagnosis remains in doubt; second even when the clinical and radiological findings make it clear that the patient suffers either from ulcerative colitis or from Crohn's disease, the biopsy does not always differentiate clearly between them (Lewin and Swales, 1966). Both aspects of this problem are being studied but only the second is reported on and discussed here.

Certain definite conclusions can be drawn from the observations. In biopsies from patients with proven ulcerative colitis, if assessment of mucosubstances is made in zones free from severe ulceration or polypoidal hyperplasia, there is a moderate or marked diminution in sulphated and acid non-sulphated mucosubstances in nearly all cases. This reduction bears a definite relationship to the degree of inflammatory cellular infiltration of the lamina propria, but not to the duration of the disease. This finding in rectal biopsies is similar to that described in colectomy specimens (Hellstrom and Fisher, 1967; Greco et al, 1967; Filipe, 1969) and may at first be thought to parallel the observation that rectal involvement is more common in colitis than in Crohn's disease. In this series, however, as in that of Hawk and Turnbull (1966), histological rectal involvement in Crohn's disease, with inflammatory change in the lamina propria, is common but reduction in mucosubstances is rare, pointing to an essential and significant difference between the two conditions. This difference, though not absolute, is of considerable help in differential diagnosis. Such a difference is perhaps not surprising since ulcerative colitis is primarily a diffuse mucosal inflammation, whereas Crohn's disease often involves the whole thickness of the bowel wall without such severe mucosal damage (Morson, 1966).

The differences in amount and type of mucosubstance seen in the different histological patterns of ulcerative colitis are not sufficient to allow these patterns to be distinguished on biochemical grounds alone, but in general it may be said that in fulminating and acute active cases of short duration the decrease in mucosubstances is slight or moderate, and that in chronic active cases it is moderate or severe, and in quiescent cases tends to revert again towards normal. These findings may be of value in assessing the progress of the disease and the effects of medical treatment.

When the mucosubstances present were considered in terms of their neutral, acid non-sulphated, and sulphated content, no loss of mucosubstance was found to differ sharply between sulphated and acid non-sulphated, though in general the mucosubstances which remained seem to be rather more acid non-sulphated than sulphated. This might be explained by the relative immaturity of newly formed cells following an alteration in cellular turnover due to damage of the regenerating zone in the fundus of the crypt (Greco et al, 1967). The differences are not sufficiently distinct to be of any diagnostic value.

In conclusion, it is felt that the addition of investigations for mucosubstances to the conventional histological assessment on rectal biopsies helps materially in the distinction of ulcerative colitis, in which mucosubstances are markedly reduced, from Crohn's disease, in which they are present in normal or slightly reduced amounts. This distinction remains in the presence of marked inflammation of the lamina propria and equivocal histology. Current studies on the
mucosubstances in non-specific proctitis may help further in the differential diagnosis of proctocolitis on rectal biopsy.

Our thanks are due to the surgical staff of the Gordon Hospital, Westminster Group, under whose care the patients were, to Miss Jean Sowden for technical assistance, to the Photographic Department of Westminster Medical School for the finished prints, and to Miss Jill Ashby for secretarial help. One of us (M.I.F.) was in receipt of a grant from the British Empire Cancer Campaign which we gratefully acknowledge.

References
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