The value of a study of the mucosubstances in rectal biopsies from patients with carcinoma of the rectum and lower sigmoid in the diagnosis of premalignant mucosa

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SYNOPSIS One hundred and twenty-one rectal biopsies from 99 patients with carcinoma of the rectum or lower sigmoid colon were investigated using a high iron-diamine-Alcian blue technique for sulphated and non-sulphated acid mucins. It was found that in normal rectal mucosa sulphomucins are the main carbohydrate component of the goblet cell mucin. This normal ‘mucous pattern’ changes in the ‘transitional’ mucosa (histological normal mucosa adjacent to carcinoma) where there is an increase of non-sulphated acid mucins concomitantly with a decrease or absence of sulphated groups in 60 to 90% of the cases according to the type of tumour. The same type of changes in mucus were observed in the ‘transitional’ mucosa surrounding adenomatous polyps and papillary adenomas; they were not marked in areas around carcinoma in situ and not observed in the metaplastic polyps. These changes seem to be in direct relationship to the grade of differentiation and invasiveness of the tumour.

The histochemical changes in the mucins seem to be in favour of a malignant potential in the so-called neoplastic polyps.

The high iron diamine-Alcian blue, because of its ‘specificity’, consistent results, and easy technique is recommended for routine use together with haematoxylin and eosin staining in the diagnosis of premalignant changes.

Early diagnosis of malignancy is of extreme importance for both pathologists and clinicians, as it implies a choice of therapeutic measures and a much greater chance of survival for the patient.

The advances in histochemical methods and electron microscopy, and, more recently, in immunohistochemistry, opened a new era by revealing earlier chemical and ultrastructural changes than those detected by the routine light microscopy.

Previous studies (Filipe, 1969; Filipe and Cooke, 1970; Filipe, 1971) have shown histochemical changes in the epithelial mucosubstances in the mucosa adjacent to carcinoma of the large intestine, which we call ‘transitional’ mucosa, compared with the normal mucosa. These results encouraged us to apply certain of these techniques to rectal biopsies from patients with carcinoma of the rectum and lower sigmoid colon and to focus our attention on the transitional mucosa in an attempt to see if information concerning the pattern of mucous secretion would be of assistance in the diagnosis of premalignant changes.

Material and Methods

All patients treated at the Gordon Hospital (Westminster Group, London) for carcinoma of the rectum or lower sigmoid colon during the period 1963-69 were reviewed. A group of 99 patients was selected and a total of 121 rectal biopsies examined. Selection was based on the following criteria:

All cases had had rectal biopsies before operation, and all had the diagnosis of carcinoma of the large intestine confirmed histologically.

All specimens were fixed either in formol saline or in 10% Baker's formol calcium and routinely embedded in paraffin. Sections were stained with haematoxylin and eosin and with the following histochemical techniques for the identification of mucosubstances: periodic acid-Schiff followed by Alcian blue at pH 2.5 (PAS-AB) (Mowry and
Morard, 1957) to distinguish between neutral (staining magenta by PAS) and acid mucins (basophilic with AB pH 2.5); high iron diamine and high iron diamine followed by Alcian blue at pH 2.5 (HID-AB) (Spicer, 1965) to separate sulphated (staining brown-black) from non-sulphated acid mucins (basophilic with AB, pH 2.5).

Results

Classification and distribution of the cases
It is important for the present investigation to know how close to the tumour the rectal biopsy was taken, as changes have been described in fragments of mucosa taken within 2 cm from the tumour (Filipe, 1969; Filipe and Cooke, 1970). An attempt was made to classify the histologically normal mucosa in the rectal biopsies examined into normal or "transitional" depending on their distances from the tumour.

Normal mucosa (22 biopsies)
This group consists of histological sections showing fragments of normal mucosa only. These pieces may come either from areas close to the tumour or far from it as it is not always possible to determine the exact site of the biopsy. Cases with some inflammatory infiltrate in lamina propria are also included.

Transitional mucosa (99 biopsies)
This group consists of histological sections in which (a) normal mucosa is found adjacent to the tumour, or (b) separated fragments of normal mucosa and tumour are present in the same section. In this case we presume that the biopsy was taken at a short distance from the tumour.

Transitional mucosa was studied in relation to the histological types of the adjacent tumour (Table).

<table>
<thead>
<tr>
<th>Carcinomas (81 biopsies)</th>
<th></th>
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<tbody>
<tr>
<td>Anaplastic .................. 2</td>
<td></td>
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<tr>
<td>Poorly differentiated .......... 11</td>
<td></td>
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<tr>
<td>Moderately differentiated .......... 37</td>
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<tr>
<td>Well differentiated .......... 16</td>
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<tr>
<td>Papillary .................. 12</td>
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<tr>
<td>Carcinoma in situ .......... 3</td>
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<table>
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<th>Polyps (18 biopsies)</th>
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<tr>
<td>Metaplastic ............. 3</td>
</tr>
<tr>
<td>Adenomatous ............ 10</td>
</tr>
<tr>
<td>Papillary .............. 5</td>
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Table Transitional mucosa in relation to histology of adjacent tumour

\[1\]
Morson (1962, 1968)

All papillary polyps showed signs of early malignant change; not all adenomatous polyps presented carcinomatous changes but as practically no histo-

chemical differences were found in the transitional adjacent mucosa to these they were all put into the same group.

Changes in the epithelial mucosubstances
Two main types of mucosubstances, sulphomucin and sialomucin, are found in the goblet cells in the rectal mucosa. Their histochemical characteristics can be briefly summarized.

Sulphomucins show basophilia with Alcian blue at pH 2.5 and stain brown-black when HID-AB and HID methods are used. They are periodate unreactive.

Non-sulphated acid components (sialomucins) have an affinity for Alcian blue at pH 2.5; they stain blue when the HID-AB method is used but no colour is obtained by the HID method; the reactivity to periodate is variable.

In normal rectal mucosa, sulphomucins are the main carbohydrate component of the mucus of goblet cells (Fig. 1).

This normal mucous pattern changes in the morphologically normal mucosa adjacent to carcinoma of the large intestine, which we call transitional mucosa. The mucosa may be thicker than the normal

![Fig. 1 Normal rectal mucosa showing predominantly sulphated mucosubstances strongly stained by the high iron diamine method. × 60.](image-url)
and with increased mucus secretion. Histochemically there is a decrease or absence of sulphomucins and a marked increase in sialomucins. These differences are clearly seen when sections are stained by the HID-AB technique.

The HID-AB technique applied to the group of biopsies with normal mucosa from patients with carcinoma of the rectum or lower sigmoid (Fig. 4) shows a normal mucous pattern in 12 cases out of 22. Changes in the epithelial mucosubstances of the types described above in the transitional mucosa are found in the other 10 biopsies (45%) in which eight (36%) present a marked increase in sialomucins with a corresponding decrease in sulphomucins (Figs. 2 and 3) and two (9%) a mixed pattern.

The findings in biopsies of transitional mucosa are shown in Figure 4. In all types of carcinoma examined, except papillary and carcinoma in situ, the epithelial mucosubstances in transitional mucosa are predominantly non-sulphated acid mucins, with a decrease or even absence of sulphated mucosubstances. The percentage of biopsies showing these changes varies between 90 and 66% according to the type of tumour, being higher in the poorly differentiated carcinomas and lower in the well differentiated group. The two cases of anaplastic carcinoma, both with sialomucins predominating, are not included in these figures. If we include the cases in which a mixed pattern is observed with an increase in sialomucins the percentage of transitional cases is even greater. The percentage of biopsies in this series with no histochemical changes varies between 9 and 6% with the type of tumour.

All three biopsies with lesions of carcinoma in situ show a mixed pattern.

The transitional mucosa in papillary carcinomas presents a different histochemical behaviour. Only four biopsies out of 12 (33%) follow the pattern described in transitional mucosa with an increase in sialomucins; another two have mixed patterns and six (50%) have a normal mucous distribution.

Regarding the transitional mucosa in polyps in patients with carcinoma of the rectum and lower sigmoid, qualitative changes in the mucosubstances of the type described are found adjacent to adenomatous and papillary polyps. These changes are more often observed with adenomas when malignant change takes place (75%) compared with 50% in

Fig. 2 Transitional mucosa. High iron diamine staining to show decreased amount of sulphated mucosubstances as compared with the normal mucous pattern (see Fig. 1) ×36.

Fig. 3 Transitional mucosa, serial section to Figure 2. High iron diamine-Alcian blue staining to show intense basophilia with Alcian blue due to non-sulphated acid mucosubstances. ×36.
benign adenomas (33% have a mixed pattern) and 60% in papillary polyps with malignant transformation. There were only five cases in this latter group, three with increased sialomucins and two having a normal mucous pattern.

No histochemical changes in mucosubstances are observed in the transitional mucosa in the three metaplastic polyps examined.

If Dukes' classification for intestinal cancers is used (Dukes, 1940) transitional mucosa adjacent to carcinomas in grade C all show an increase in sialomucins as described above. This does not happen in Dukes' A and B grades. The grade A carcinomas, three biopsies out of nine (33%), show a normal pattern. In grade B six cases present a normal pattern and three have a mixed pattern out of a total of 27 (33%). The relationship between invasiveness and changes in mucosubstances is not so apparent between grades A and B as it is between these two on the one hand and C on the other.

The duration of symptoms and the sex and age of the patient are factors which seem to have no influence on the changes described.

Discussion

From the results presented it seems evident that the carbohydrate component in the goblet cells changes qualitatively and quantitatively in the morphologically normal mucosa next to carcinoma of the large intestine as compared with the normal mucosa. These changes, confirmed by chemical analysis (Filipe and Cooke, 1970), consist mainly of an increase in sialomucins, and the percentage of biopsies in which this increase is found varies according to the type of tumour.

The group with a higher percentage of cases showing these changes includes all types of carcinoma from the anaplastic to the well differentiated, the exception being the papillary variety.

A direct relationship seems to exist between the histochemical patterns and the degree of differentiation and invasiveness. The only exception in the above group is observed in papillary carcinoma in which deviations from the normal mucous pattern do not occur so often. This apparently different behaviour could possibly be explained by the early stage of invasion of most of the tumours examined. The findings in carcinoma in situ, where a mixed pattern with sialomucins and sulphomucins was present in the three cases examined, are in agreement with this interpretation.

The study of the transitional mucosa in polyps found in patients suffering from carcinoma of the
The value of a study of the mucosubstances in rectal biopsies

large intestine seemed a matter of interest. According to Morson (1962, 1968), the only intestinal polyps which are precancerous are the adenoma, papillary adenoma, and villous papilloma which he classifies as epithelial neoplastic lesions. Other varieties of non-neoplastic polyps are hamartomas, inflammatory, and unclassified. The last includes 'metaplastic' polyps.

The histochemical study of the mucins revealed a different pattern in the neoplastic polyps as compared with the metaplastic polyps and the normal mucosa. This different histochemical aspect applies not only to the glandular structures which constitute the 'polyp' itself (Filipe, 1969) but also to the corresponding transitional mucosa, as the present work demonstrates.

The histochemical findings described in the transitional mucosa and in the glandular structures of the polyps raise certain questions.

RELATIONSHIP BETWEEN NEOPLASTIC AND METAPLASTIC POLyps
These are histochemically different, the latter having a similar mucous pattern to the normal mucosa. This fact is further evidence for their being classified and regarded as separate entities. The metaplastic polyps accordingly should be considered non-neoplastic lesions with no tendency to undergo malignant change (Morson, 1962, 1968).

RELATIONSHIP BETWEEN ADENOMATOUS POLYPS AND PAPILLARY ADENOMAS
The most acceptable theory is that the papillary adenoma is a 'growth' variant of the adenomatous polyp and that it is essentially the same pathological process (Enterline, Evans, Mercado-Lugo, Miller, and Fitts, 1962; Morson, 1968). Apparently there are no significant histochemical differences in the mucous secretion in these two lesions and from that point of view we can consider them as one entity.

RELATIONSHIP BETWEEN ADENOMATOUS AND PAPILLARY ADENOMA AND CARCINOMA
Several authors have stated the facts in favour (Rider, Kirsner, Moeller, and Palmer, 1954; Grinnell and Lane, 1958; Morson, 1962, 1968; Bussey, Wallace, and Morson, 1967; Enterline and Arvan, 1967) or against (Ackerman, 1964) the evidence of the malignant potential of these lesions. The histochemical study of the mucins does not at the present provide an answer to the problem. However, the changes observed in the transitional mucosa in a high percentage of these cases suggest that either the neoplastic polyps are precancerous lesions or at least the apparently normal mucosa adjacent to them is subject to the same type of stimulus as that adjacent to carcinoma.

Conclusions

The percentage of cases showing an increase in sialomucins was only 36% in the group of 'normal' rectal biopsies. This does not invalidate the previous results; it is possible that some fragments were taken from areas not sufficiently close to the tumour. But even so, we think that an increase in the accuracy of diagnosis is significant.

Are these changes specific for the mucosa adjacent to carcinoma and neoplastic polyps of the large intestine, or can they be found with other diseases and in other sites? How significant are these changes for diagnostic purposes?

We can say, based on purely histochemical grounds, that these changes were only consistently found in the lesions described in this work. Previous histochemical studies of the mucins in non-neoplastic conditions of the human large intestine (Filipe, 1969; Johansen and Kay, 1969; Filipe and Dawson, 1970) did not show modification in the mucins or else such modifications were of a different type, quantitative rather than qualitative. However, our findings are not specific for large intestinal neoplasias (Lev, 1965; Esterly and Spicer, 1968; Korhonen and Mäkelä, 1968).

Although other methods have revealed various deviations from the normal in the areas adjacent to neoplastic lesions (Wattenberg, 1959; Nairn, Fothergill, McEntegart, and Richmond, 1962; Cole and McKalen, 1963; Lipkin, 1966, 1970; Czernobilsky and Tsou, 1968), we think that the histochemical study of the mucins offers at present more specific and consistent results and the advantage of an easy technique for routine use. Therefore we suggest that the application of the HID-AB technique for sulphomucins and sialomucins is useful in the diagnosis and evaluation of premalignant mucosa.

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