

A histochemical method of differentiating lower gastrointestinal tract mucin from other mucins in primary or metastatic tumours

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SYNOPSIS Epithelial mucins of the normal terminal ileum, caecum, colon, and rectum of man are unique in that they alone exhibit staining following the periodate-borohydride/KOH/PAS technique. Application of this technique enables one to differentiate those mucin-producing metastases arising from adenocarcinoma of the lower gastrointestinal tract from those arising elsewhere, and may occasionally be useful in determining the site of the primary tumour when it is in doubt. Furthermore, it was found to be especially useful in distinguishing between primary adenocarcinoma of the lung and metastases in the lung from adenocarcinoma of the lower gastrointestinal tract.

We have shown that, in certain defined areas of the gastrointestinal tract, there is an increase in periodic acid-Schiff (PAS) reactivity following treatment with potassium hydroxide (KOH), and that this effect is due to the removal of O-acyl esters from the side chains of sialic acids in epithelial mucins (Culling *et al*, 1971, 1974; Reid *et al*, 1973). Originally, detection of this increase in PAS reactivity required a comparison microscope but we have shown that periodate oxidation followed by reduction with sodium borohydride abolished the normal PAS reactivity of tissue, and the subsequent application of the KOH/PAS technique ensures that only its effect is seen (Reid *et al*, 1973).

This paper describes the use of the periodate-borohydride KOH/PAS technique as a means of distinguishing between mucins secreted by metastases which have arisen from adenocarcinoma of the lower gastrointestinal tract and those mucins which have been secreted by normal or malignant cells from other tissues. This technique should be especially valuable where the metastases have grown more rapidly than the primary lesion, the former being the presenting complaint.

Materials and Methods

NORMAL HUMAN TISSUES

Routinely formalin fixed, paraffin processed blocks of the following normal tissues were examined:

parotid, submaxillary, and submandibular glands, liver, thyroid, bronchus, lung, eye, seminal vesicle, aorta, testis, prostate, ovary, uterus, cervix, fallopian tube, kidney; and stomach, small intestine, caecum, colon, and rectum. In addition, there were the numerous relatively normal tissues (for example, liver, lung, breast, ovaries, lymph nodes) containing tumours or metastases.

NEOPLASTIC TISSUES

Formalin fixed, paraffin processed blocks from 89 primary adenocarcinomas of colon (49), rectum (2), caecum (2), stomach (10), small intestine (1), lung (20), breast (3), bile duct (1), and ovary (1) were examined together with 177 blocks of their metastases.

MICROSCOPICAL EXAMINATION

Specimens were examined without the observer being aware of the site of the primary tumour.

Staining methods (Culling, 1974)

1 Haematoxylin and eosin (H and E) was used to establish the diagnosis and identify tissues.

2 Periodic acid-Schiff reaction (PAS) was used as a control.

3 Periodate borohydride/potassium hydroxide/PAS technique (PB/KOH/PAS) was used to demonstrate the KOH/PAS reaction in isolation.

Paraffin sections of routine formol-calcium or buffered formalin fixed tissues may be used.

METHOD

1. Sections were brought to water, and
2. oxidized with 1% aqueous periodic acid at room temperature for 60 minutes, and
3. washed in water.
4. They were then reduced in sodium borohydride as follows:

Solution A

2.45% boric acid in distilled water; this solution may be stored in the refrigerator.

Solution B

1.89 g sodium borohydride dissolved in 167 ml of distilled water; this solution must be made immediately before use.

Slides were placed in a glass (or stainless steel) slide holder (of the bottomless variety, not a coplin jar). This was placed in a container which held 300 ml yet was narrow enough for the sections to be completely covered by the addition of 100 ml of solution A; this container must be kept cool, by surrounding it with crushed ice. Solution B was then added gradually over a period of 30 minutes and the sections were left for an additional hour; this step should be carried out in a fume hood. They were then washed in running water for 10-15 minutes.

5. Sections were treated in 0.5% potassium hydroxide in 70% alcohol at room temperature for 30 minutes,
6. washed in 70% alcohol, then in water; and
7. stained by the periodic acid-Schiff (PAS) technique,
8. dehydrated, cleared, and mounted in HSR.

RESULTS

Mucin showing any red coloration is interpreted as being KOH/PAS positive, provided the slide treated by the PB/PAS technique (see below) is unstained. Optionally, sections may be lightly counterstained with haematoxylin, or tartrazine in cellosolve, which will visualize the tissue and will aid the orientation of the section; we preferred the former.

RAPID PERIODATE-BOROHYDRIDE/KOH/PAS TECHNIQUE

This method, which utilizes a modification of Lillie and Pizzalato's borohydride aldehyde blocking method, can be used as a routine method. However, if a particular section treated by the periodate borohydride/PAS technique (see below), which is used as a negative control, shows any red colour, then the long method (above) should be used.

1. Sections were brought to water and then treated with 1% periodic acid for 30 minutes,

2. washed in running water for 10 minutes, and
3. treated in 0.1% sodium borohydride in 1% disodium hydrogen phosphate for 30 minutes,
4. slides were washed in water.

Steps 5, 6, 7, and 8 and results as in method above.

PERIODATE BOROHYDRIDE/PAS TECHNIQUE (PB/PAS)

This technique was used to ensure that normal PAS reactivity had been abolished. The method consists of steps 1, 2, 3, and 4 (above) followed by the routine PAS technique and should be performed in parallel with the above method (PB/KOH/PAS). Any staining present in this section will indicate that the periodic acid-borohydride treatment is incomplete due to either (1) insufficient time of oxidation with periodic acid; or (2) incomplete reduction by sodium borohydride. The substitution of simple Schiff reagent for 30 minutes in place of a full PAS method will help to determine the cause since a positive result by the latter indicates that the reduction by sodium borohydride is incomplete.

Results

PATHOLOGICAL TISSUES

These are summarized in the table and it will be seen that, of the 89 tumours and 177 metastases examined, in no instance was a metastasis positive by the KOH/PAS technique when the primary tumour was negative. With one single exception, only those tumours that had arisen in the caecum, colon or rectum gave a positive KOH/PAS reaction, and on no occasion did a metastasis from a negative tumour give a positive result.

One primary tumour of the stomach was itself KOH/PAS positive and gave rise to metastases (3) which were also KOH/PAS positive. The primary

Site of Primary Tumour	KOH/PAS Positive		KOH/PAS Negative	
	Primary Tumour	Metastases	Primary Tumour	Metastases
Colon	35	55	—	—
Colon	—	—	14	23
Caecum	2	4	—	—
Rectum	2	4	—	—
Stomach ¹	1	3	—	—
Stomach	—	—	9	26
Small intestine	—	—	1	2
Lung	—	—	20	42
Breast	—	—	3	4
Bile duct	—	—	1	6
Ovary	—	—	1	8

Table Results of KOH/PAS staining on 89 mucin-producing adenocarcinomas and their 177 metastases, subdivided by the site of the primary tumour

¹See text

tumour showed the histological features of both colloid and adenocarcinoma.

In the table it will be seen that there was no KOH/PAS positive mucin (or other material) in either the primary tumours, which had arisen from tissues other than the gastrointestinal tract, or their metastases. This was felt to be significant since a biopsy of a solitary metastasis from several of these cases might have presented a problem to surgical pathologists asked to determine the site of the primary tumour since it would have been difficult to rule out the possibility of the lower gastrointestinal tract being the site.

It will be of interest that on two recent occasions we have been able to direct the attention of surgeons to the colon and rectum as primary sites based upon the presence of KOH/PAS positive mucin in biopsies of the metastases.

NORMAL TISSUES

With the exception of a very pale red coloration seen in the liver, normal tissues from sites other than the lower gastrointestinal tract did not stain by the periodate-borohydride/KOH/PAS technique. A slight exception to this statement is the fact that myelin sheaths are positive but they present no difficulty in interpretation. Mucin in sections of terminal ileum (up to 30 cm from the caecum) may show a weak KOH/PAS effect, and metastases from tumours arising in this area might be expected to give a similar result although such an assumption needs to be tested.

Discussion

The KOH/PAS effect has been shown by us both histochemically (Culling *et al*, 1974) and chemically (Reid *et al*, 1975) to be due to the presence of O-acylated sialic acids in an epithelial mucin which is only produced by cells of, or cells derived from, the lower gastrointestinal tract of man. Further, we have demonstrated that this effect can be seen in isolation by first abolishing the normal PAS reactivity of tissue sections by oxidation of the existing vicinal diols (1:2 glycol groups) with periodic acid, followed by the reduction of the engendered aldehydes with sodium borohydride.

In this study it was found that only mucin gave a positive result by this technique and that, with one exception, such mucin had been produced either (a) by normal epithelial cells in the caecum, colon or rectum (or the terminal ileum immediately adjacent

to the caecum) or (b) in the primary tumour, or in metastases from such a primary tumour which had arisen in this area.

The features of importance which arise from these observations are, in our opinion:

(1) Mucin-producing metastases from adenocarcinoma of the lower gastrointestinal tract can be identified as such when the KOH/PAS reaction is positive, such identification being of importance when the site of the primary tumour is unknown. On two occasions at the Vancouver General Hospital we have been able to provide surgeons with such information.

(2) Mucin-producing primary adenocarcinoma of the lung (which will be KOH/PAS negative) can easily be distinguished from mucin-producing metastases from adenocarcinoma of the lower gastrointestinal tract when the primary tumour is KOH/PAS positive, which occurred in 70% of those we studied.

(3) The discovery that 30% of adenocarcinomas of the defined area of the lower gastrointestinal tract are KOH/PAS negative may prove to be of clinical significance. It may be surmised that this group of tumours arises from a different cell type or that a greater degree of dysdifferentiation of the cells has taken place. A study is now being undertaken to determine the possible significance of this finding.

Supported by Medical Research Council of Canada, Grant # 4375

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