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REVIEW ARTICLE

MUCINS AND GASTROINTESTINAL MALIGNANCY

A new approach to the interpretation of biopsies

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SUMMARY

Mucous secretion in the gastrointestinal tract shows regional variations: neutral mucins predominate in the gastric mucosa, sialic acid-rich compounds in the small intestine and sulphated mucins in the large intestine; glycoprotein synthesis also varies with cell maturation from crypt to surface epithelium. Changes in these normal mucous patterns have been observed in carcinoma and particularly in the non-neoplastic adjacent mucosa: A) In the stomach extensive and severe intestinal metaplasia with increased sulphomucin secretion is often associated with carcinoma; B)In the small intestine alteration in the proportion of various sialic acids and the presence of sulphated material are found in areas adjacent to carcinoma; C) In the large intestine harbouring carcinoma sialomucins become predominant with decrease or absence of sulphated radicals. Alterations in glycoprotein synthesis have been implicated in malignancy and we believe that the changes described above may represent a feature of malignant transformation and thus be of value in the interpretation and possibly detection of early cancer.

INTRODUCTION

Pathologists are often faced with diagnostic problems which are difficult or impossible to solve by routine histological methods, but which may be resolved by histochemical techniques.

In this review we propose to discuss some problems associated with the diagnosis and control of gastrointestinal malignancy. The aims are:

a) to detect early malignant change
b) to assess patients at risk of developing cancer
c) to clarify the relationship between the biological behaviour of tumours and prognosis.
d) to determine the origin of the primary cancer.

We believe that a multi-disciplinary approach to gastrointestinal cancer, involving cell pathology, immunology, biology and biochemistry are needed to understand the histogenesis and the biological nature of this disease.

A variety of tumour markers have been investigated with little practical value at present (Coombs 1978).

Alterations in carbohydrate metabolism have been consistently associated with malignant transformation and there is cummulative biochemical data to illustrate the changes in sialic acids noted in this process (Currie and Bagshawe 1968; Warren et al 1972; Van Beek et al 1973; Isselbacher 1974). Most of this work has been carried out in the cell surface glycoproteins of transformed cells. More recently, investigations on secretory glycoproteins (mucins) in normal gastrointestinal epithelium and their variations in neoplasia, have aroused great expectations (Filipe and Branfoot 1974, 1976;

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Kim et al 1974; Culling et al 1977a; Gorman and LaMont 1978; Rogers et al 1978; Filipe 1979; Jass and Filipe 1979).

Mucins of the human gastrointestinal epithelium (G. I.) are glycoproteins formed by a protein backbone with attached side chains of sugar residues often with a terminal sialic acid. In some, ester sulphates are also present.

Histochemically, the mucins are divided into neutral and acid, the latter being either rich in sialic acid (sialomucins) or sulphate groups (sulphomucins). Sialomucins can be further subdivided according to the proportion of N-acetyl or O-acetyl derivatives of sialic acid in the molecule and whether or not they are labile to neuraminidase digestion. A battery of histochemical techniques is available to separate these different groups (Pearse 1968). However, in our experience, the main types of G. I. mucins can be easily identified by a combination of the methods shown in Table 1, which are used routinely in our laboratory.

Table 1

Histochemical identification of mucins

METHODS / MUCINS	PAS*	PB/KOH/PAS**	HID-AB
NEUTRAL	+(RED)	O	O
SIALOMUCINS			
N-acyl	+(RED)	O	+(BLUE)
O-acyl	+(RED)	+(RED)	+(BLUE)
SULPHOMUCINS	O	O	+(BROWN/BLACK)

KEY: +Positive; O negative
* PAS=Periodic Acid Schiff. Gastric biopsies are also stained with ABpH2.5-PAS.
** PB/KOH/PAS=Periodate-Borohydride/Saponification/PAS (Culling et al 1974). Not used as a routine technique

NOTE: All these methods are carried out in formalin-fixed tissues, routinely embedded in wax.

GASTRIC MUCOSA

Early diagnosis is an essential prerequisite for the successful treatment and control of gastric cancer. While advanced gastric cancer has a poor prognosis, the 5-year survival rate after surgical treatment for early gastric cancer is 95-100 % (Johansen 1976; Morson 1977a).

The role of the pathologist is the careful screening of gastric biopsies for detection of a) early gastric cancer and b) precancerous lesions.

Collaboration between endoscopist and pathologist is important and a good preparation of the biopsy material is essential if the maximum information is to be obtained. The samples should be correctly orientated by placing the tissue, mucosal face upwards, on pieces of frosted glass. The whole is then immersed in fixative. For wax embedding the tissue is removed from the glass, turned on its side and embedded as usual. This technique allows sections to be cut at right angles, thus producing a biopsy with longi-

tudinally open full length glandular tubules, abuting on to the surface epithelium and resting its base against the muscularis mucosae. At least three or four serial sections at two levels should be cut, stained with Haematoxylin-eosin, PAS, ABpH2.5-PAS, (Pearse 1968), and HID-AB (Spicer 1965) for mucin identification. This procedure applies to all gastrointestinal biopsies.

The mucous secreting cells of the *normal* gastric epithelium, including foveolae, cardiac and pyloric glands and the mucous neck cells, secrete neutral mucins almost exclusively. Traces of acid mucins (sialomucin and possibly sulphomucins) can be seen at the base of the pits and mucous neck cells in the body and occasionally in the lower pit in the antrum. In the oesophageal-gastric junction both types of acid mucins may be found.

Early gastric cancer is defined as a carcinoma which has not yet extended beyond the submucosal layer of the stomach wall (Johansen 1976). The histological features of intramucosal carcinoma may be present in the biopsy and easily recognised. However, in cases of undifferentiated or signet ring cell types of carcinoma, groups of isolated malignant cells may not be detectable on H. E. stained sections and mucin stains are helpful.

The *precancerous lesions* in the stomach are the adenomas and intestinal metaplasia (I.M.)

Intestinal metaplasia has been shown to be associated with gastric cancer (Morson 1955; Laurén 1965; Johansen 1976; Day and Morson 1978a) but it is also common in benign conditions such as chronic atrophic gastritis and peptic ulcer (Morson and Dawson 1972). Although the majority of gastric cancers seem to arise from areas of I.M., not all do so. An origin from the normal gastric epithelium cannot be excluded (Johansen 1976).

To investigate the distribution and variants of intestinal metaplasia in carcinoma and non-malignant gastric disease, we carried out a detailed histological and histochemical study in carefully prepared gastrectomy specimens. These were collected fresh, opened along the greater curve, pinned down on cork, and fixed in formalin. All specimens were either photographed or a sketch was made of the outlines and the macroscopical lesions. Blocks were taken from the lesions and adjacent mucosa. Strips of mucosa were also cut along the length of the lesser and greater curves and from other areas where appropriate, and coiled up in *swiss rolls*. Serial scetions were stained with H.E. and mucin stains. (Jass and Filipe 1979).

Three variants of I.M. according to histological features and mucus secreting patterns have been described (Ming et al 1967; Iida et al 1978; Teglbjaerg and Nielsen 1978; Jass and Filipe 1979). The classical type of I.M. resembles small intestine and secretes sialomucins. Another variant shows features of colonic mucosa with its high goblet absorptive cell ratio, secretion of sulphomucins and presence of O-acetyl derivatives of sialic acid, which are found in the colonic but not in the small intestinal mucins (Culling et al 1975; Filipe and Fenger 1979). A third *Intermediate* or *Incomplete* type of I.M. contains elements of both gastric and intestinal epithelia. Goblet cells secrete either sialo or sulphomucins or a mixture of the two. The intervening columnar cells reveal the presence of neutral, sialo and sulphomucins in varying proportions.

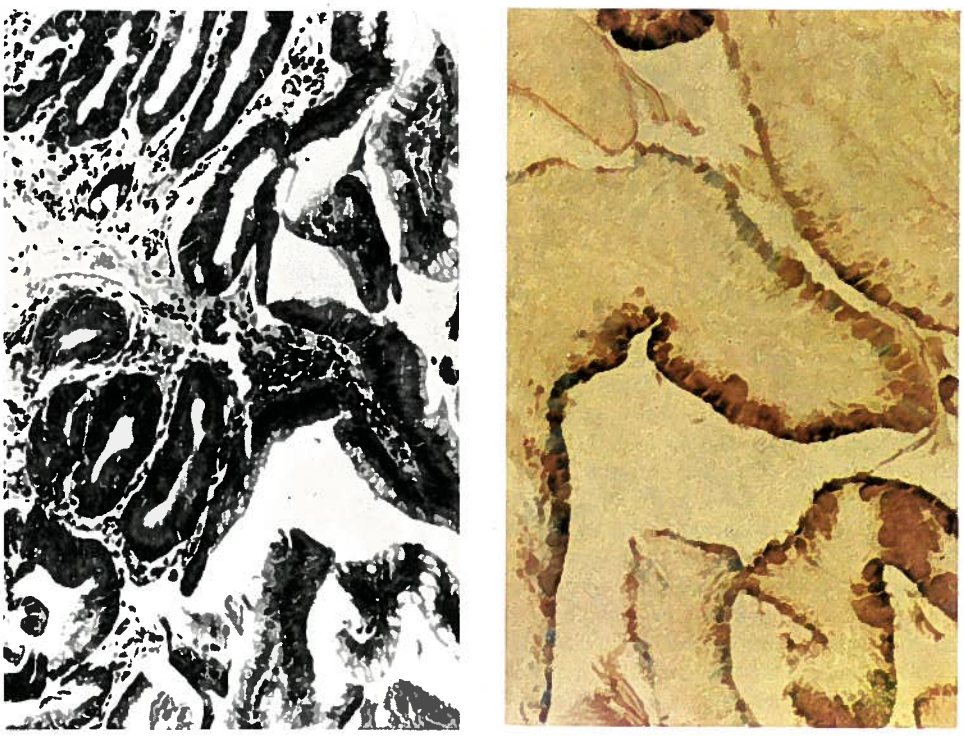
We observed that the classical type of I.M. is more commonly found in peptic ulcer, while the other two types, particularly those with marked secretion of sulphomucins show, in our series, a significant association with carcinoma (Figs 1 a and b). Lev in 1966 reported the presence of sulphomucins in mucosa adjacent to gastric carcinoma and recently Teglbjaerg and Nielsen (1978) described a *colonic type* of I.M. more frequently found in stomachs bearing intestinaltype carcinomas. The presence of

sulphomucins in gastric mucosa harbouring carcinomas deserves attention as it may be related to foetal sulphoglycoprotein demonstrated by Häkkinen in the gastric juice of cancer patients (Häkkinen et al 1968; Häkkinen 1974).

Further evidence that this variant of I.M. might be premalignant is its presence in adenomatous polyps and adjacent metaplastic epithelium and absence from hyperplastic polyps (Jass and Filipe in preparation) which have minimal malignant poencial (Ming 1977; Kozuka et al 1977).

The finding of I.M. in a gastric biopsy has little practical value because it is common to a variety of gastric lesions. On the other hand, the identification of a subgroup more consistently related to cancer could have important implications in the selection of patients at risk of developing cancer and thus in need of careful follow up. Our studies suggest that *incomplete* and *colonic* type of I.M. secreting sulphomucins may have a higher malignant potential.

Gastric *carcinomas* are commonly classified into intestinal type (53 %) and diffuse type (33 %), the rest being unclassified (Laurén 1965). It has been suggested that the aetiology and pathogenesis of these two types may be different on the criteria of age distribution, severity of I.M. and prognosis (Laurén 1965; Johansen 1976; Day and Morson 1978a). Efforts to correlate the biological behaviour of these two types of carcinoma with mucous secreting patterns have so far failed. The amount and composition of mucins vary from case to case and in different areas within the tumour (Gad 1969; Jass and Filipe 1979).



a b

Fig. 1 — *Gastric biopsy. a) H and E staining shows carcinoma and areas of intestinal metaplasia. b) I.M. secretes sulphomucins (broun). HID-AB. a* × *75; b* × *96*

SMALL INTESTINAL MUCOSA

Tumours of the small intestine, particularly carcinomas, are rare. This may be the reason why so little attention has been paid to mucus secretion in the small intestine in contrast with the colon and stomach.

Structure and biological function of glycoproteins are intimately related. A better knowledge of its composition will give us insight of its role in the physiology and pathology of the small intestine. It has been shown that mucus secretion varies with cell differentiation and in disease states (Culling et al 1977b; Lance et al 1978; Filipe and Fenger 1979). Thus, it is possible that changes im mucin composition may alter its physicochemical properties and lead to an unprotected mucosa more vulnerable to injury.

In the *normal* duodenum, jejunum and ileum, goblet cells secrete a PAS positive material which contains neutral as well as sialomucins. Sulphomucins are absent. The proportion of the different types of mucins produced vary along the crypts and villi, with an increasing amount of sialomucins towards the villus top. (Filipe and Fenger 1979).

It is interesting to note that in the human foetal small intestine, mucin droplets around the 7th week contain only PAS positive neutral mucins and that sialomucins, labile to sialidase, appear later in the development (Lev et al 1972). It is thus tempting to correlate the variations in the mucins along the crypts and villi with cell differentiation: neutral mucins produced by the immature cells in the crypts, with sialomucins being synthesized as cells mature and migrate towards the villi.

Regional differences are also apparent and suggest a gradient of change in the mucin composition with an increasing content of O-acylated sialomucins (PB/KOH/PAS effect) and the presence of sulphated esters in a few goblet cells in the normal terminal ileum, as if to establish a transition to colonic mucins which contain a larger proportion of O-acylated sialomucins and sulphated material (Culling et al 1977b; Filipe and Fenger 1979).

Carcinomas of the small intestine, like the gastric and the colonic discussed below, do not show in our series any specific pattern of mucus secretion. They are often non-secretory or contain a mixture of neutral and acid mucins in varying proportions from case to case. However, the non- involved mucosa adjacent to carcinoma shows particular features which differ from the normal mucosa in the greater amount of mucus produced, associated with an increased proportion of O-acylated derivatives of sialic acid and the presence of sulphomucin (Filipe and Fenger 1979).

COLONIC MUCOSA

In the *normal* colonic mucosa, a large proportion of mucins are sulphated (sulphomucins) (Fig. 2). As in the small intestine, the composition of the goblet cell mucin varies with its level in the crypt as well as in the different segments of the colon. In the left colon (and rectum) sulphomucins predominate in the lower half of the crypt with sialomucins seen in the upper crypt and surface epithelium. In the right colon it is not uncommon to find sialomucins in the lower third of the crypt with sulphomucins occupying the upper two thirds. A higher content of O-acylated variants of sialic acid is also noted in the left colon and rectum (Filipe 1971 a, b; Filipe and Branfoot 1976; Filipe 1979).

Colorectal cancer is one of the commonest forms of malignant disease in Europe and North America (Morson and Dawson 1972). Cancer control depends on a) early diagnosis and b) identification of precancerous lesions.

Early diagnosis: small established carcinomas can often be detected now by advanced radiological and colonoscopic techniques, but quite often tiny endoscopic biopsies show *histological normal mucosa only.* There is thus a need for new methods to increase the accuracy of detection of early malignant transformation in biopsies, before currently recognised cytological criteria of atypia are met. The study of mucins is encouraging in this field.

Fig. 2 — *Normal rectal mucosa stained with HID-AB to show predominance of sulphomucins (brown)* × *300*

Mucosa from specimens harbouring carcinoma reveals a sepctrum of morphological and secretory changes, some of which fall short of accepted criteria of dysplasia (Filipe and Branfoot 1974; 1976).

We use the term *transitional* to describe these areas of mucosa where altered mucus secretion is found in the absence of morphological atypia. This abnormal mucus pattern is characterised by increased sialomucins and decreased or absent sulphated material in contrast with normal colonic mucosa where sulphomucins predominate (Fig. 3 a-d). Further histochemical and biochemical characterisation of sialomucins in normal, *transitional* and carcinoma tissues demonstrated the presence of varying proportions of N-acetyl and O-acetyl derivatives of sialic acid with the values for *transitional* mucosa graded between those from normal and tumour (Dawson et al 1978; Rogers et al 1978).

The important question is whether the changes described in *transitional* mucosa are primary or secondary to the tumour growth and if primary, whether they represent an early feature of malignant transformation or a non-specific cellular response to unknown stimuli.

At present we believe that they are a primary effect and may represent a prepolypoid phase of an adenomatous growth. Some observations strongly suggest such a relationship.

Thus, histologically, this *transitional* mucosa is either normal or more often thicker with elongated branched crypts and dilated goblet cells (Fig. 3 a, d) resembling the features Kozuka (1975) described as grade 1 in his grading of adenomatous polyps. Our own ultrastructural studies where a gradation of changes could be traced from *transitional* mucosa to adenoma and carcinoma also support this view (Mughal and Filipe 1978).

A histological and histochemical study of 100 consecutive specimes of large bowel resected for carcinoma, from which strips of mucosa were cut from the entire length of each specimen and coiled up in *swiss rolls* revealed that these changes, though more marked in the mucosa adjacent to carcinoma are also found in isolated patches distant from tumour. This suggests a primary rather than local secondary effect of tumour growth. Furthermore, the extend of mucin changes, invasiveness of carcinoma and prognosis seem to be related. (Filipe and Branfoot 1976; Greaves et al 1979).

Precancerous lesions: these are adenomas, familial polyposis and ulcerative colitis.

Adenomas: Most cancers of the colon arise from adenomatous polyps but it is clear that not all adenomas will fulfil their malignant potential in a normal lifetime. (Morson 1974). Why do some evolve to cancer, some remain static and others even regress? Can one predict which polyp will become malignant? Morson's criteria for assessing the malignant potential of adenomas is based on size, growth pattern and the degree of epithelial atypia. (Morson 1977 b; Day and Morson 1978 b). Yet a polyp after many years may fail to show malignant transformation with which it was credited according to these criteria.

Distinct patterns of mucus secretion are found in metaplastic (hyperplastic) and neoplastic (adenomatous) polyps. In the former, mucins are predominantly sulphated like those in the normal mucosa and the adjacent mucosa shows no change. In contrast, in the adenomas the amount of mucin secreted decreases with increasing dysplasia and is usually a mixture of neutral and acid mucins. The mucosa around these shows often a predominance of sialomucins similar to the *transitional* area adjacent to carcinoma (Makela et al 1971; Filipe and Branfoot 1976). Our studies in isolated polyps and in familial polyposis coli, could not confirm the results presented by Goldman and Ming (1968) of a relationship between the types of mucin secreted and the degree of differentation of the adenomas. Recently, an association between malignancy in villous adenomas and low levels of O-acylated sialic acids has been reported (Culling et al 1977a).

In face of the failure to find means of predicting which adenomas will evolve to cancer, the present cancer prevention policy is to remove all polyps. The management of the patient depends on the accuracy of the pathologist's diagnosis, which can only be achieved if adequate material is provided. If possible, the whole polyp sould be removed, to include stalk (if pedunculated) and normal adjacent mucosa. The polyp should be correctly orientated and sections cut at various levels. The identification of the different types of polyp is of utmost importance and the report should also refer to the growth pattern, degree of dysplasia, presence or absence of invasion through the muscularis mucosae and whether or not excision has been complete.

Familial polyposis coli: This is a rare hereditary disease, where hundreds of adenomas are present throughout the large bowel and the patients will develop carcinoma if left untreated.

Cancer prevention in this disease is dependant on treating the adenomas (by colectomy resection) before they become malignant (Bussey 1975; 1978)

Mucin studies in 23 colectomy specimens from cases of familial polyposis coli failed to show any consistent pattern of mucus secretion either in the polyp or in the intervening non-malignant mucosa. A tendency towards non-sulphated mucus secretion has been observed, more apparent in the left colon than in the right and more marked in areas adjacent to carcinoma, but with no apparent relatioship with the degree of dysplasia of the adenomas (Filipe et al in preparation).

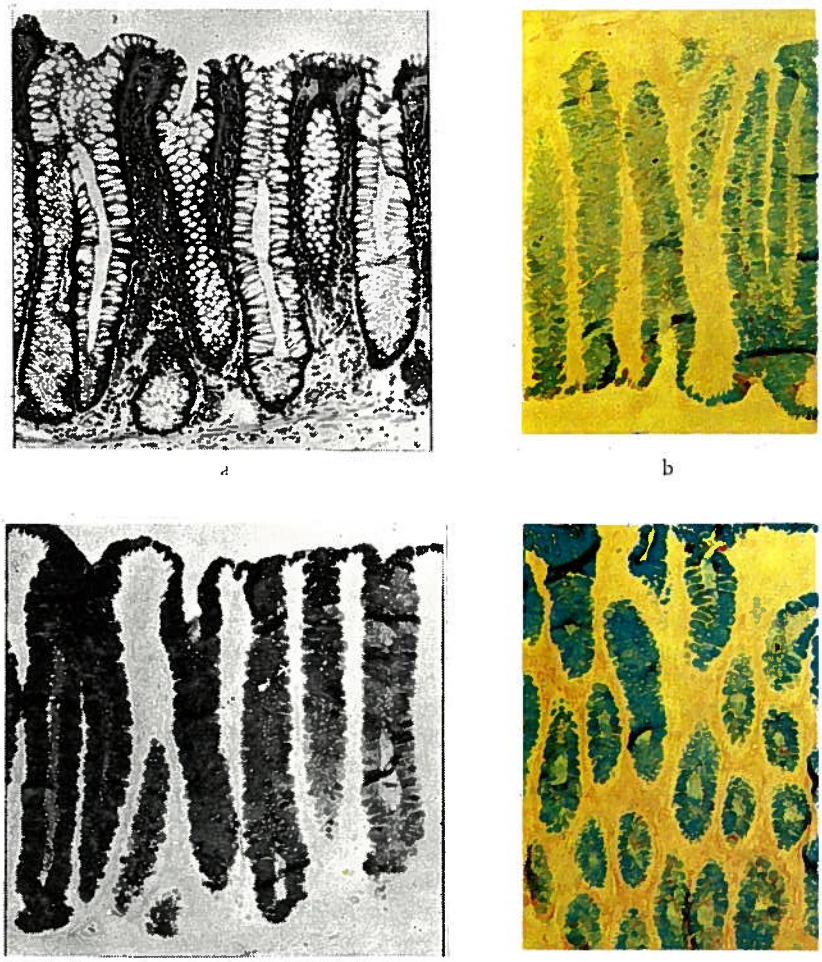


Fig. 3 — *Rectal biopsies from a patient with a clinical diagnosis of ? carcinoma of the rectum. Ist biopsy: (a and b). a) H and E stained section shows features of «Transitional» mucosa with taller branched crypts and dilated goblet cells but no epithelial atypia. No tumour was found in the sections. b) The HID-AB stained serial section shows marked increase of sialomucins (blue) as compared with the normal mucus pattern in Fig. 2. In view of these findings a 2nd biopsy (c and d) was performed c) H and E stained section shows carcinoma (arrows) and adjacent «Transitional» mucosa. d) HID-AB stained serial section shows predominance of sialomucins (blue), and absence of brown stained sulphate material. a and c* × *54; b and d* ×*58*

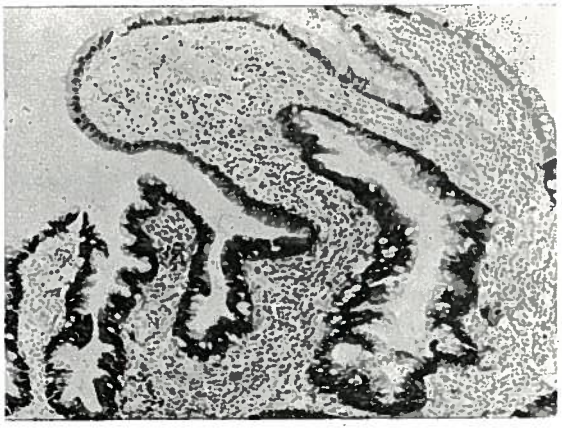
Ulcerative colitis: The incidence of carcinoma in ulcerative colitis is low, but greater than in the general population.

The tumour tends to develop at an earlier age. The problem here is the identification of patients at higher risk of developing cancer. This group consists of patients suffering from total colitis, for more than 10 years, particularly if the onset of the disease occured before the age of 25. The incidence of carcinoma in this group is around 1 % (Riddell 1976).

The other group at risk consists of patients treated for ulcerative colitis by colectomy and ileorectal anastomosis, where there is the possibility of carcinoma arising in the retained rectum. The incidence of rectal carcinoma in this group is 5.9 % and the prognosis is poor (Baker et al 1978).

For both groups there is the need of careful follow-up. Rectal and colonoscopic biopsies should be done at regular intervals and there is thus an increasing need for the recognition of precancer. Yet, its diagnosis is not always simple.

The general features of precancer are agreed and its value has been attested (Morson and Pang 1967; Yardley and Keren 1974; Riddell 1976). However, its patchiness, absence from the rectum when carcinoma is found elsewhere in the bowel, danger of



a

b

Fig. 4 — *Precancer in ulcerative colitis. a) H and E stained section shows villous mucosa with frank epithelial dysplasia. b) HID-AB staining reveals predominance of sialomucin. a × 45 b × 45*

over diagnosis and lack of precise definition particularly in the presence of inflammation, remain a problem (Riddell 1976; Lennard-Jones et al 1977; Riddell et al 1978).

Our preliminary studies, encourage us to believe that mucin changes may add another dimension in the diagnosis of precancer in biopsies and be of help in the assessment of high risk patients.

We have found *transitional* mucosa with predominance of sialomucin secretion in specimens of ulcerative colitis resected for carcinoma. Mucin stains seem useful in distinguishing reactive hyperplasia where the pattern of secretion is normal from true dysplasia where sialomucins predominate. They are also useful in distinguishing the reactive form of villous mucosa where again mucus secretion is normal, from the precancer villous mucosa where sialomucins are increased (Fig. 4 a, b). In contrast colonic biopsies from colitis patients in remission phase show a normal mucus pattern with predominance of sulphomucins (Fig. 5) (Filipe and Branfoot 1976; Filipe 1977).

Carcinoma: Like those of gastric and small intestinal origin, colonic carcinomas when secretory, show marked variation in the composition of mucus. (Fig. 6). No pattern of mucus secretion has emerged which could be related to the histological grade of the tumour. (Gad 1969; Korkonen et al 1971; Filipe and Branfoot 1976).

Fig. 5 — *Ulcerative colitis in remission showing a normal mucous pattern with presence of sulphomucins. Compare with Figs. 2 and 4. HID-AB* × 75

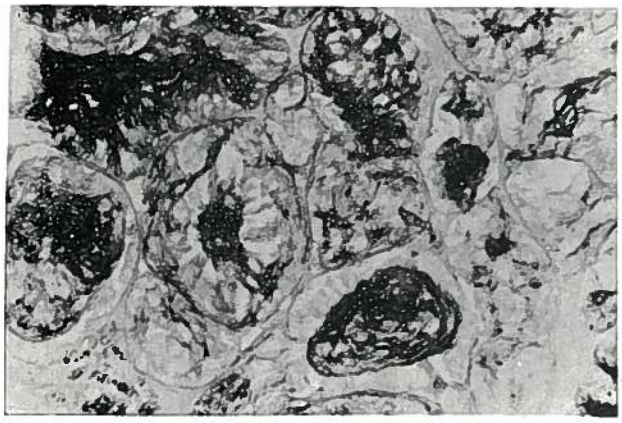


Fig. 6 — *Colonic adenocarcinoma secreting a mixture of sialo-(grey) and sulphomucins (black). HID-AB* × 75

IDENTIFICATION OF ORIGIN OF METASTATIC CARCINOMA

The composition of mucin secretion by tumour cells along the gastrointestinal tract, as described above, reveals no histochemical characteristics which could be related to organ of origin or histological type. Attempts to identify the origin of adenocarcinomas have not been successful (Johnson and Helwig 1963; Cook 1973). This is not surprising since carcinomas arise from undifferentiated or immature cells, probably originating from a common stem cell throughout the gastrointestinal tract, capable of synthesising glycoproteins in various stages of completion.

The demonstration by Culling et al (1975) of O-actyl derivatives of sialic acid in the lower gastrointestinal tract carcinomas and its absence from all others could be a useful means of differential diagnosis (Figs. 7 and 8).

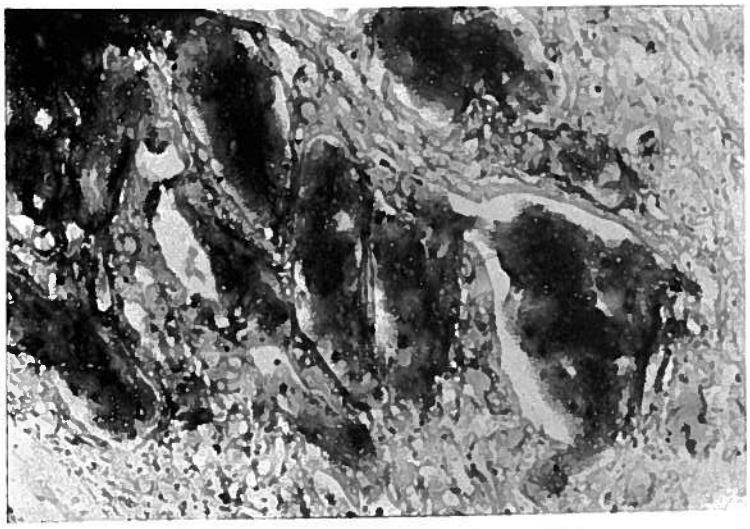


Fig. 7a — *Colonic mucus-secreting adenocarcinoma. Strong PAS-reaction* × 90

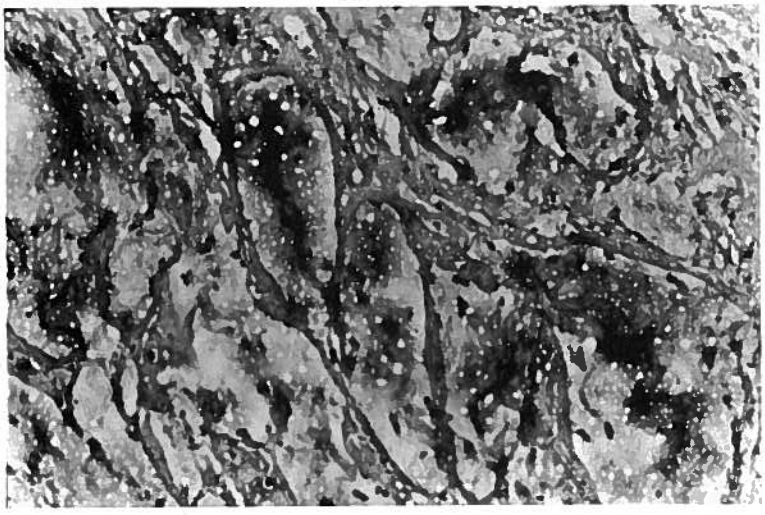


Fig. 7b — *Colonic mucus-secreting adenocarcinoma. After PB/KOH treatment, PAS-reaction persists, suggesting the presence of O-acyl derivatives of sialic acid.* × 90

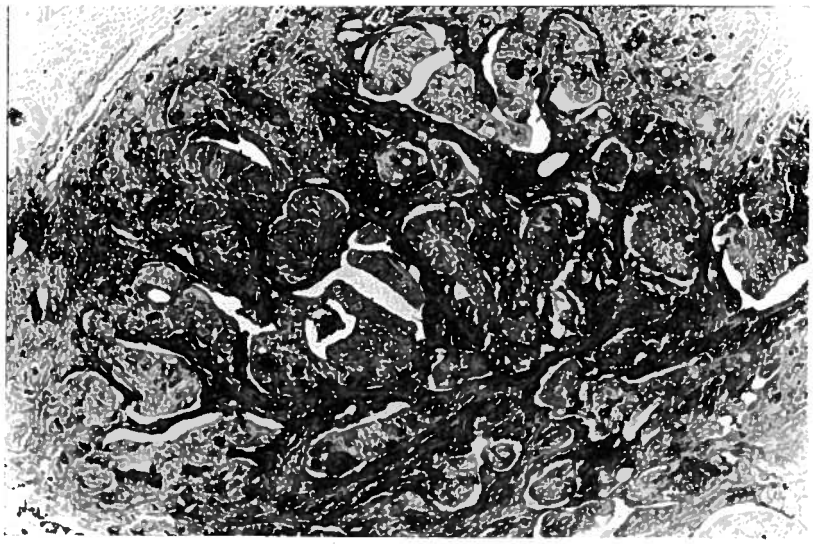


Fig. 8a — *Small intestine. Mucus-secreting adenocarcinoma. Strong PAS-reaction* ×90

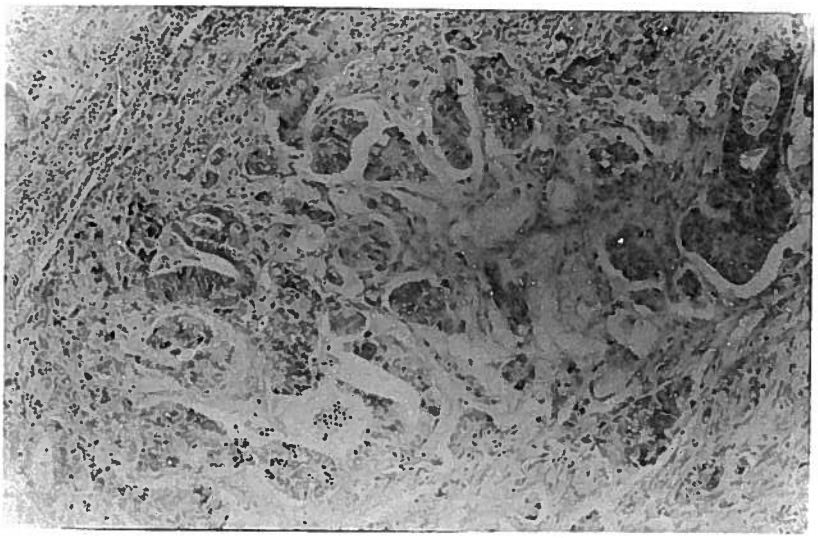


Fig. 8b — *Small intestine. Mucus-secreting adenocarcinoma. After PB/KOH treatment, PAS-reaction is totally abolished, suggesting absence of O-acyl derivatives of sialic acid. To compare with Fig 7* × 90

RESUMO

As secreções mucosas do tracto gastrentestinal apresentam uma variação regional: enquanto que as mucinas neutras predominam na mucosa gástrica, os compostos ricos em ácido siálico são mais abundantes no intestino delgado e as mucinas sulfurosas concentram-se sobretudo no colon; a síntese das glicoproteínas varia também paralelamente à maturação celular desde as criptas até ao epitélio de superfície.

Determinadas alterações na normal secreção de mucinas têm sido observadas em casos de neoplasia, atingindo uma maior expressão na mucosa peri-neoplásica: A) No estômago, a metaplasia intestinal extensa com aumento de secreção de sulfomucina associa-se frequentemente ao carcinoma; B) No intestino delgado, as alterações na proporcionalidade entre os diversos ácidos siálicos e a presença de compostos sulfurosos, são detectadas na proximidade dos carcinomas; C) No colon portador de carcinoma, as sialomucinas são predominantes em detrimento dos radicais sulfato que podem mesmo estar ausentes.

As alterações na síntese das glicoproteínas têm sido implicadas na génese das neoplasias e, na nossa opinião, os achados acima descritos representam uma etapa da transformação maligna revestindo-se, desta maneira, duma enorme importância na interpretação e detecção do carcinoma precoce.

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