

# Abnormal goblet cell glycoconjugates in rectal biopsies associated with an increased risk of neoplasia in patients with ulcerative colitis: early results of a prospective study

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**SUMMARY** A group of 18 patients with stable ulcerative colitis involving the entire colon for at least eight years was subjected to a biopsy of normal appearing rectal mucosa and followed prospectively over four years for the development of either dysplasia or cancer. Goblet cell glycoconjugate structure was examined in the rectal biopsies using fluorescein conjugated lectins. At the beginning of the study, 13 of the 18 patients had abnormalities of goblet cell mucin or cytoplasmic glycoconjugates in the rectal biopsies. Dysplasia subsequently developed in six and carcinoma in one of these patients. Among the five patients with normal lectin binding studies in the initial rectal biopsies, colonic dysplasia has subsequently developed in one. The abnormalities seen in the rectal goblet cells resembled in part those previously seen in immature and neoplastic colonic cells. The dysplastic tissues all contained the form of mucin which has been found in other neoplastic colonic tissues. This preliminary report after four years of prospective study suggests that abnormalities of glycoconjugate structure may be associated with, and may precede, neoplastic events in the setting of chronic ulcerative colitis.

Patients with chronic ulcerative colitis have an increased risk of developing colonic cancer, especially when the disease is longstanding and involves the entire colon.<sup>1-3</sup> These tumours are hard to identify at an early stage because the manifestations of ulcerative colitis may be difficult to distinguish from those of cancer, and gross mucosal distortions produced by the disease may mask a small neoplastic focus from detection by the radiologist or endoscopist.<sup>4</sup> Diffuse abnormalities of the colon have therefore been sought that might enable the identification of malignant or premalignant changes at a potentially curable stage.

It has long been appreciated that a reduction in the amount of goblet cell mucus is characteristically seen in rectal biopsies from patients with ulcerative colitis.<sup>5,6</sup> In addition, qualitative differences have been detected between the colonic mucus of healthy subjects and patients with ulcerative colitis.<sup>7</sup> Our laboratory has found that fluorescein isothiocyanate

(FITC)-conjugated lectins are useful as probes of the carbohydrate structure of mucins, and that changes in the lectin binding characteristics reflect the state of differentiation and the presence of malignant transformation in colonic epithelial cells.<sup>8,9</sup>

It was decided, therefore, to study the goblet cell glycoconjugates in rectal biopsies of patients with stable, chronic ulcerative colitis in an effort to detect early signs of neoplastic change. A group of patients was followed prospectively for four years after an initial rectal biopsy from each had been studied with FITC-lectins. Those patients who subsequently developed neoplastic changes in the colon came predominantly from the group in which abnormalities of lectin binding had been detected at the beginning of the study.

## Methods

### PATIENTS

A group of 18 patients with chronic ulcerative colitis at high risk to develop neoplasia was selected, each

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with total ulcerative colitis for a minimum of eight years (range 8–34 years, median 20 years), and disease that was stable and free of neoplasia at the beginning of the study in 1979. All patients underwent rectal biopsies from a randomly selected site in the rectum 2–10 cm from the anus. The biopsies were fixed in Bouin's solution and embedded in paraffin. The biopsies were examined for pathological diagnosis and the presence of dysplasia (by RHR), coded, and sent to the University of California for study with FITC-lectins. None of the tissues was acutely inflamed. Five micron sections were cut, serially rehydrated, and incubated with 50  $\mu$ l of a commercially (Vector Laboratories, Burlingame, CA) obtained FITC-lectin solution at 200  $\mu$ g/ml, pH 7.1 for a period of 20 minutes followed by rinsing and mounting.<sup>8</sup> Eight different FITC-lectins were used (Dolichos biflorus agglutinin (DBA), soybean agglutinin (SBA), peanut agglutinin (PNA), Bauhinia purpurea agglutinin (BPA), Ricinus communis agglutinin-1 (RCA-1), wheat germ agglutinin (WGA), concanavalin A (ConA) and Ulex europeus agglutinin-1 (UEA-1)) (see Table 1). Coincubation with inhibitory hapten sugars confirmed the labelling specificity of the lectins.<sup>8</sup> The slides were examined under fluorescence microscopy by two

independent observers (CRB and PL) and the labelling of goblet cell glycoconjugates was scored from 0 (absent) to 4+ (maximum fluorescence), and compared with findings previously obtained from normal colonic mucosa.<sup>8,10</sup> Agreement in labelling was reached on all tissues. The patients were followed clinically for three years (by BL). Colonoscopy was performed annually and biopsies taken at 10 cm intervals from caecum to rectum to screen for neoplasia. Most of the patients were treated with sulphasalazine, and some required the intermittent use of steroids.

The code was broken in 1982 and the results of the lectin studies were compared with the clinical outcome for each patient. Additional tissues were obtained for lectin studies from four of the patients who developed dysplasia (A, C, M, and N).

The data were analysed using a  $\chi^2$  contingency table.

## Results

### DATA OBTAINED USING FITC-LECTINS ON THE INITIAL RECTAL BIOPSIES

The results of the labelling with four of the lectins (ConA, RCA-1, UEA-1, and WGA) were essentially the same as those found in the normal colon<sup>8,10</sup> (see Table 2). With the four other lectins (DBA, SBA, PNA, and BPA), however, differences were seen (Table 3). A marked reduction in the labelling of goblet cell mucin occurred in 5/18 with FITC-DBA and 8/18 with FITC-SBA (Figs. 1–3). A total of 9/18 showed a reduction in labelling with either DBA or SBA, which require exposed GalNAc residues for binding. FITC-PNA produced an unusual form of labelling in 11/18 of the biopsies in which glycoconjugates in the supranuclear region were labelled (Figs. 4 and 5). In only one biopsy was the goblet cell mucin also labelled by PNA. An increase in labelling of goblet cell mucin (compared with superficial biopsies from the normal colon)<sup>8,10</sup> was seen in 4/18 using FITC-BPA. Increased labelling of BPA was seen only in biopsies which also had abnormalities of lectin binding using DBA, SBA or

Table 1 Lectins used to study glycoconjugates in ulcerative colitis

Lectin	Major sugar binding specificity	Binding to mucin in normal colonic biopsies (0–4+)	Comments
DBA	GalNAc—	4+	Markers of well differentiated goblet cells in the upper part of the colonic crypt
SBA	GalNAc—	4+	
PNA	Gal <sup>1-3</sup> <sub>P</sub> GalNAc—	0	Marker of cancer-associated colonic mucin
BPA	Gal—	0–2+	Preferentially label mucin from goblet cells in the lower part of the colonic crypt
RCA	Gal—	0–2+	
WGA	GlcNAc— NeuNAc—	4+	Labels human colonic mucins under all conditions
ConA	Mannose—	Variable	May represent the presence of serum glycoproteins in mucus
UEA	Fucose—	Usually 0	

DBA = Dolichos biflorus agglutinin, SBA = soybean agglutinin, PNA = peanut agglutinin, BPA = Bauhinia purpurea agglutinin, RCA = Ricinus communis agglutinin-1, WGA = wheat germ agglutinin, Con A = concanavalin A, UEA = Ulex europeus agglutinin-1, GalNAc = N-acetylgalactosamine, Gal = Galactose, GlcNAc = N-acetylglucosamine, NeuNAc = N-acetylneuraminic acid (sialic acid).

Table 2 Labelling of biopsies with ConA, RCA, UEA, and WGA, showing no significant difference between ulcerative colitic and normal colon<sup>10</sup>

Lectin	Normal colon	Ulcerative colitis
ConA	Variable; usually 0–2+	All 0–1+
RCA	0–2+	0–2+
UEA	Usually 0	0
WGA	4+	4+

Table 3 FITC-lectin labelling data using DBA, SBA, PNA, and BPA – qualitative abnormalities detected in ulcerative colitis lectin labelling characteristics

Patient	Duration of disease (yr)	Lectin labelling characteristics				Clinical outcome during study
		DBA	SBA	PNA	BPA	
A	34	‡	‡	*	§	Dysplasia
B	32	‡	‡	n	n	
C	27	n	n	n	n	Colectomy (benign stricture)
D	25	n	n	*	n	
E	23	n	n	n	n	
F	22	n	n	*	n	
G	21	n	n	n	n	
H	21	n	n	n	n	Dysplasia; colectomy
I	19	n	n	n	n	
J	18	n	‡	*	n	
K	14	‡	‡	*	§	
L	13	n	‡	*	n	Dysplasia
M	13	n	‡	*	n	Dysplasia
N	13	n	n	*	n	Dysplasia
O	11	‡	‡	*	§	
P	11	n	‡	n	n	
Q	9	‡	n	*	n	Dysplasia
R	8	n	n	†	§	Adenocarcinoma

n = labelling identical to that in normal colon, \* = labelling occurs in the supranuclear glycoconjugates (?Golgi region), † = labelling in the supranuclear region and goblet cell mucin, ‡ = a marked reduction in or loss of labelling compared with the normal colon, § = substantially increased compared with normal colon.

PNA. A total of 13/18 of the biopsies showed at least one abnormality of lectin binding; abnormalities with more than one lectin were found in 9/18; 4/18 showed a single aberration, and in 3/4 of these cases the single abnormality occurred with PNA (Table 4).

#### CLINICAL OUTCOME

All 18 patients have been followed clinically for the four year study period. During this time, serial colonoscopic biopsies have revealed the development of dysplasia (determined independently by

Table 4 Summary of abnormal labelling of goblet cell glycoconjugates

Lectin	Labelling in normal colon	Labelling in ulcerative colitis colon
DBA	4+	Reduced to 0–2+ in 5/18
SBA	4+	Reduced to 0–2+ in 8/18
PNA	0	Supranuclear labelling in 11/18 Goblet cell mucin labelling in 1/18
BPA	0–2+	Increased labelling to 3–4+ in 4/18

RHR) in six patients (A, H, L, M, N, and Q; see Tables 3 and 5). One patient underwent a colectomy for a stricture which proved to be benign (patient C). One patient was found to have a carcinoma of the transverse colon two years into the study period (patient R).

Six of the seven patients who developed dysplasia or carcinoma came from the subset of 13 in whom abnormalities of goblet cell glycoconjugates had been detected in the initial biopsy at the beginning of the study. Five of the six had abnormal labelling with more than one lectin. The patient who underwent a colectomy for a stricture (patient C) had no neoplasia upon careful examination of the resected specimen, and his rectal biopsy had shown normal lectin binding. Dysplasia has developed in the caecum of one patient who had normal lectin binding studies at the initial biopsy (patient H). Because of progression of the dysplasia, he underwent a colectomy and no carcinoma was found in the resected specimen. One rectal biopsy showed labelling of supranuclear glycoconjugates and goblet cell mucin by FITC-PNA (Fig. 6). This patient (R) developed carcinoma two years after the initial rectal biopsy. No patient died or developed fulminant disease during the study period.

$\chi^2$  analysis of the results using a two by two contingency table to test the association between the presence of PNA-binding glycoconjugates in the rectal biopsy and neoplasia gave a  $\chi^2=2.91$ , which corresponds to  $0.10 > p > 0.05$ .

#### LECTIN STUDIES ON DYSPLASTIC TISSUES

Colonic biopsies that showed dysplasia from four

Table 5 Development of neoplasia during clinical follow up

Patient	Neoplastic development	Location	Time interval (yr)
A	Dysplasia (mild)	tc	2
H	Dysplasia (mild)	caecum, ac, dc	2
	Dysplasia (severe)	(severe only in caecum)	2.5
L	Dysplasia (mild)	rectum	1
	No dysplasia	—	2
	Dysplasia (mild)	rectum	3
M	Dysplasia (mild)	rectum	3
N	Dysplasia (borderline)	rectum	initial biopsy
	Dysplasia (mild)	rectum	2
	Dysplasia (mild)	rectum, tc	3
Q	Dysplasia-associated mass	tc	3
	Dysplasia (mild)	rectum	1
R	Adenocarcinoma, with metastases	tc	2

tc = transverse colon, ac = ascending colon, dc = descending colon.

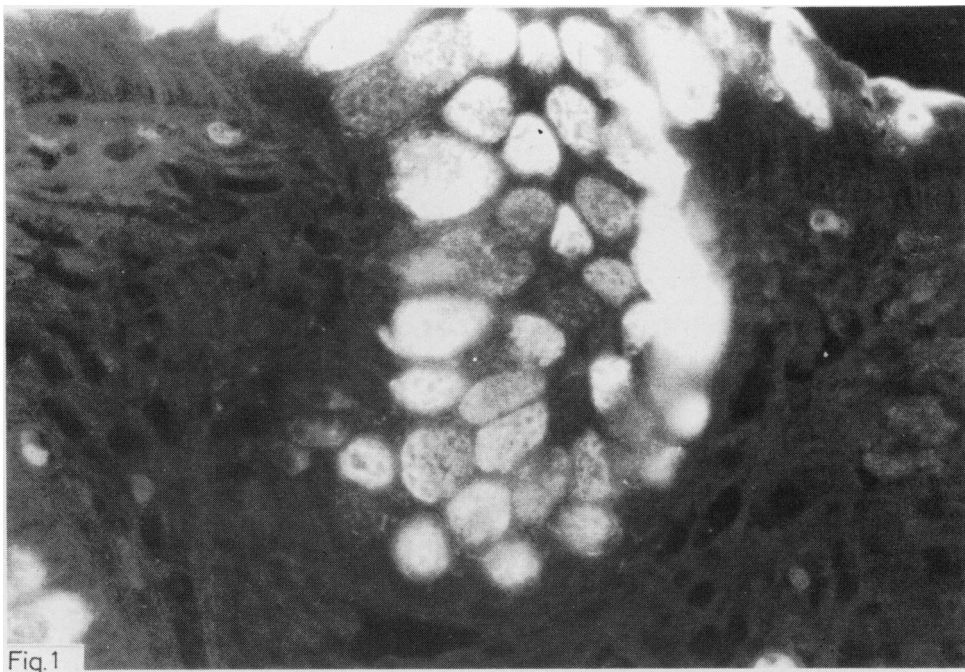


Fig. 1 FITC-SBA labelling of rectal biopsy material from patient I. Intense (4+) fluorescent labelling of the goblet cell mucin similar to that seen in the normal colon. ( $\times 125$  original magnification).

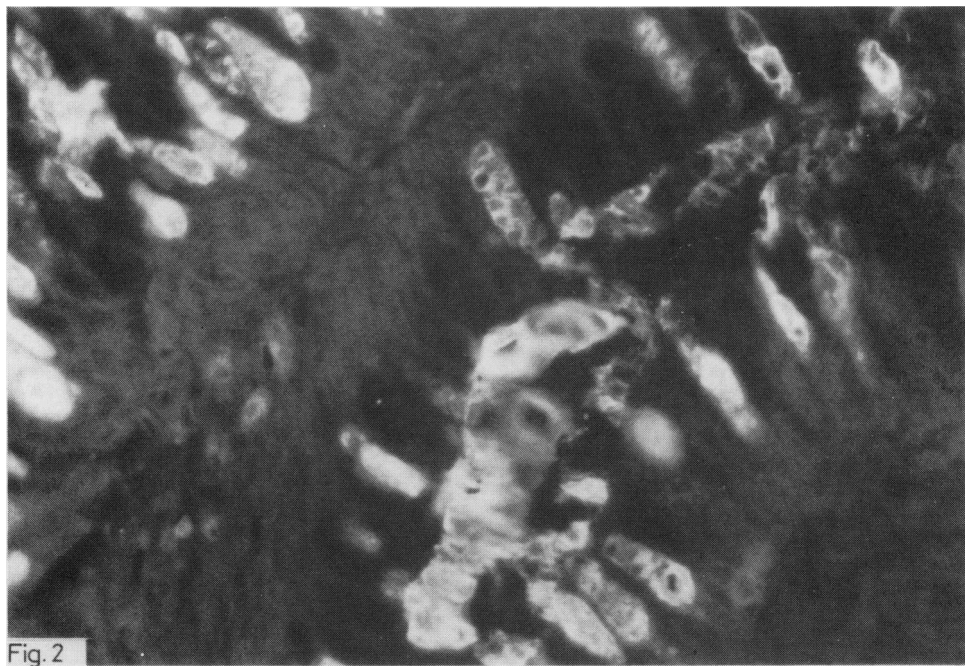


Fig. 2 FITC-DBA on rectal biopsy material from patient J, showing failure of labelling in some goblet cells, but with preservation of intense labelling in several remaining goblet cells. ( $\times 125$  original magnification).

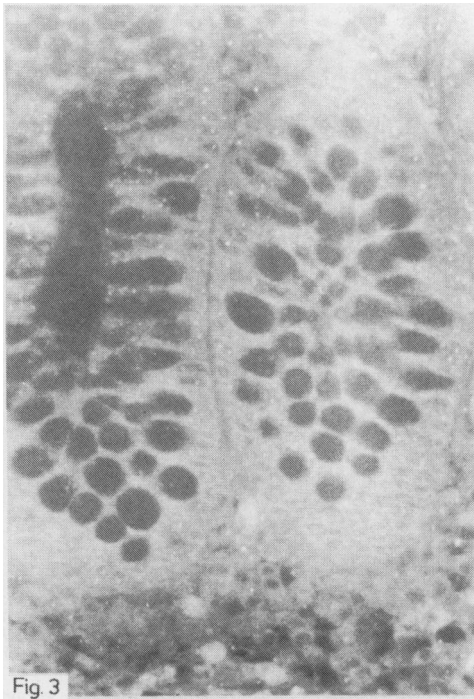


Fig. 3



Fig. 4

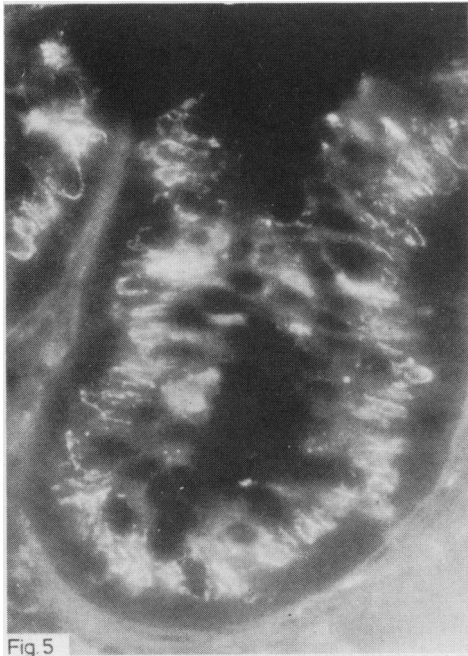


Fig. 5

Fig. 3 FITC-SBA applied to rectal biopsy from patient A, showing failure of lectin to label any goblet cell mucin. ( $\times 125$  original magnification).

Fig. 4 FITC-PNA labelling of a non-dysplastic rectal biopsy from patient A. Note linear arrangement of fluorescence, which corresponds to cytoplasmic domain between basally situated nucleus and apical portion of the cell, perhaps Golgi region. Mucus itself is not labelled. ( $\times 125$  original magnification).

Fig. 5 FITC-PNA labelling of rectal biopsy from patient R. Again, the supranuclear region is labelled. ( $\times 125$  original magnification).



Fig. 6 FITC-PNA on rectal biopsy of patient R (also in Fig. 5); note intense labelling of goblet cell mucin in this field. This was the only initial rectal biopsy to show labelling of goblet cell mucin with FITC-PNA, and this patient developed cancer two years later. ( $\times 125$  original magnification).

patients (Table 5) were studied with lectins, and PNA labelled either supranuclear glycoconjugates or goblet cell mucin in each case. In one case, patient N, a 'dysplasia-associated mass lesion' was studied, revealing extensive labelling of the goblet cell mucin (Fig. 7).

### Discussion

Colorectal cancer is one of the most serious complications of chronic ulcerative colitis, and the excess risk begins after approximately eight years of disease, rising progressively with time to 50–60% for patients who have disease of more than 30 years standing.<sup>1–3</sup> We have previously reported the consistent presence of mucin which binds DBA but not PNA in normal specimens of colon. This observation has been confirmed in a larger study of normal colonic tissues (manuscript in preparation). Our data show that in a small cohort of patients with ulcerative colitis, after 8–34 years of disease (mean 20 years) the majority have developed qualitative abnormalities of goblet cell glycoconjugates in randomly obtained samples of rectal mucosa. These changes partially resembled those seen in immature

goblet cells deep in the colonic crypt of the normal colon<sup>10</sup> (diminished binding by DBA and SBA, and increased binding by BPA), suggesting that the surface epithelial cells may be less mature in ulcerative colitis.

Six of our 13 patients with glycoconjugate abnormalities in the initial rectal biopsy have developed either dysplasia or cancer, but because of the same sample size a longer period of time is required to evaluate the predicative power of finding abnormal mucins in a rectal biopsy. Statistical analysis of the association between PNA-binding to the rectal biopsy and the subsequent development of neoplasia revealed that the association fell short of significance ( $0.10 > p > 0.05$ ). Although the group under study is a high risk subgroup of colitic patients (all with chronic pancolitis for 8–34 years), more time is necessary to identify all those who will eventually develop neoplasia. Because of the small cohort size and four year period of follow up, for the result to have been highly significant either more cases of neoplasia would have been required from the 'abnormal rectal mucin' group, or no cases of neoplasia would have been required from the 'normal rectal mucin' group. The dysplasia which



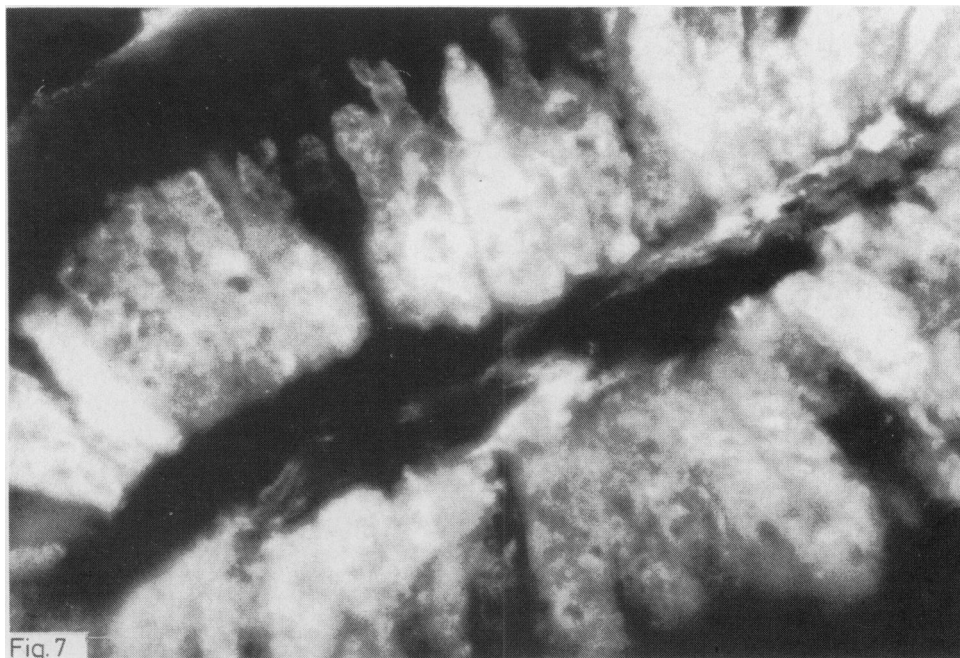


Fig. 7 FITC-PNA labelling of dysplastic biopsy specimen obtained from patient A two years after initial rectal biopsy. Note extensive labelling of goblet cell mucin, similar to that seen in Fig. 6. ( $\times 200$  original magnification).

occurred in a patient from the latter group was worst in the caecum and spared the rectum. This suggests that although the changes in goblet cell glycoconjugates may precede neoplasia in a given location, a single biopsy may be inadequate to assess the neoplastic risk of the entire colon.

Biochemically, the reduction in binding of DBA and SBA to goblet cell mucin indicates that there are fewer terminal, non-reducing GalNAc residues in the mucin oligosaccharide chains, which may be because of incomplete glycosylation of glycoproteins in the immature colonic epithelial cells.<sup>10</sup> Analysis of the membrane glycoproteins from colonic cancer has previously revealed a marked reduction in total carbohydrate content, ABO blood group activity and glycosyltransferase activity compared with those from normal colon.<sup>11</sup> Data from our laboratory obtained on mucins from colorectal carcinoma and colonic epithelium remote from cancers were consistent with the concept that the oligosaccharide chains are abnormal, and possibly incompletely synthesised in the transformed cells. This phenomenon might also be expected to result in the deletion of immunochemical characteristics expressed on the peripheral portion of the carbohydrate chain, thereby exposing structures normally hidden in the inner portion.<sup>10</sup>

The appearance of glycoconjugates which were bound by PNA is also seen in the mucin of colonic cancer as well as in adenomatous polyps and transitional mucosa.<sup>8,9</sup> The presence of PNA binding in the supranuclear region but not in the goblet cell vacuole leaves the identity of these glycoconjugates open to question. The lectin may be labelling immature mucins in the Golgi region. An analysis of the binding specificity of PNA<sup>12</sup> and a consideration of the composition of a typical mucin-type oligosaccharide chain<sup>13</sup> suggest that PNA would bind to a structure only found in the inner portion of the chain, supporting the concept that these changes reflect a defect in glycosylation of the mucin molecule. In support of this, Clamp *et al* have reported that the oligosaccharides extracted from the mucus of colectomy specimens from patients with ulcerative colitis and Crohn's disease of the colon contained only half as many carbohydrate units as were found in the mucus from normal colons obtained at necropsy.<sup>7</sup>

Using histochemical techniques, non-specific abnormalities of goblet cell mucin have been reported in the rectal biopsies of patients with ulcerative colitis.<sup>14</sup> Ehsanullah *et al* have recently reported excess sialomucins in approximately half of the rectal biopsies of patients with ulcerative colitis,

as well as most of the biopsies containing dysplasia, which parallels our own experience.<sup>15</sup> This group and others have reported similar changes in transitional mucosa,<sup>16</sup> adenomatous polyps,<sup>17</sup> and familial polyposis coli.<sup>18</sup> Other data, however, have suggested that these changes are not necessarily specific for neoplastic activity.<sup>19, 20</sup>

The identification of a marker of colonic premalignancy in randomly obtained mucosal specimens has long been a goal of investigators in inflammatory bowel disease. Our data describe changes in the glycoconjugates of rectal mucosa in a small group of patients who have been studied over a short period of time. Until more patients are examined and the period of follow up is extended, the conclusions must remain preliminary. Abnormalities of mucin, however, are seen in dysplastic tissues which resemble those seen in cancer. Similar changes are seen in non-neoplastic rectal biopsies of many patients who later develop colorectal neoplasia in this setting. As dysplasia develops in a patchy manner in the colon, it may be necessary to use lectin binding studies on biopsy specimens taken throughout the colon and rectum to improve the sensitivity of the technique. A longer period of follow up is necessary to determine the specificity of these findings, and we will continue to follow this cohort prospectively. We feel that evaluating goblet cell mucin and cytoplasmic glycoconjugates with lectins is a promising approach in the assessment of neoplastic risk in ulcerative colitis.

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