Changes in composition of mucin in the mucosa adjacent to carcinoma of the colon as compared with the normal: A biochemical investigation

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SYNOPSIS Fifteen surgical specimens from patients with carcinoma of the colon and rectum were studied. Scrapings from normal mucosa distant from the tumour and from macroscopically normal mucosa adjacent to the tumour ('transitional') were used for chemical estimation of hexosamines, sialic acid, and proteins. The presence of hexosamines and sialic acid was confirmed in both normal and transitional mucosa. Transitional mucosa showed increased levels of total hexosamines and sialic acid as compared with the normal and this was accompanied by an increase in neuraminidase-sensitive sialic acids. The present data have been compared with previous histochemical and autoradiographic studies and it is suggested that the changes described in the transitional mucosa are transformations representing an early stage of carcinogenesis.

Burdette (1970) proposed that the mucosa adjacent to a tumour could be histologically normal but still have undergone 'pre-malignant' changes acquiring an increased potential for malignant transformation which might be reflected in changes in ultrastructure and histochemical or chemical properties compared with the true normal mucosa.

The demonstration of such changes in this adjacent mucosa, which we term 'transitional', would be valuable in defining the true infiltrative volume of a tumour, in assessing the 'at-risk' cases for follow up and possibly in evaluating the prognosis (Filipe, 1972). Thus both Langvad (1968) and Lewis, Morson, February, Hywel Jones, and Misiewicz (1971) reported changes in the lactate dehydrogenase isoenzyme patterns of such mucosa in the absence of morphological change, whilst histochemically demonstrable modification of the mucin composition of peri-tumour areas have been described by Esterly and Spicer (1968) in the gallbladder, by Lev (1966) in the stomach, and by Filipe (1969, 1971b) in the colon. Immunofluorescence studies have also shown that modifications occur in areas around the tumour in carcinoma of the colon (Nairn, Fothergill, McEntegart, and Richmond, 1962; Gold and Freedman, 1965; Krupey, Gold, and Freedman, 1968; Nairn, 1969) as have studies with ^3H -

thymidine (Lipkin and Quastler, 1962; Deschner, Lewis, and Lipkin, 1963; Lipkin, 1965, 1966, 1970; Lipkin, Deschner, and Troncale, 1970).

Histochemical variations in the mucins of the large gut have been previously reported by one of us (Filipe 1969, 1971a and b) and we now report their further characterization by the chemical analysis of the glycoproteins of normal and transitional mucosa of human large intestine.

Material and Methods

PREPARATION OF TISSUES

Fifteen surgical specimens were obtained by surgical resection from patients suffering from carcinoma of the large intestine and processed immediately. In every case scrapings of mucosa were taken from normal mucosa distant from the tumour and from apparently normal mucosa immediately adjacent to the tumour (transitional mucosa), as previously described (Filipe, 1969; Filipe and Cooke, 1970). The tissues were frozen in liquid nitrogen and stored at -70°C until used. Pieces of frozen tissue, approximately 200 mg wet weight, were allowed to thaw for a few minutes at room temperature, minced and digested with 1.5 ml of papain digestion solution (Antonopoulos, Gardell, Szirmai, and De Tyssonsk, 1964; Filipe, 1971b) in a water bath at 65°C overnight (approximately 20 hours). The papain

digests were then centrifuged at 800 rpm for 10 min and the supernatant was dialysed against distilled water in Visking tubing (8/32 in. in diameter) for 24 hr at 4°C. After dialysis aliquots were used for chemical analysis.

CHEMICAL ANALYSIS

Total hexosamines were estimated by the Elson-Morgan method modified by Boas (Rimington, 1955; Boas, 1953). Sialic acid was liberated either by neuraminidase or acid hydrolysis and then determined by the thiobarbituric method (Aminoff, 1961). For neuraminidase hydrolysis the papain digests were incubated with a half volume of enzyme (*Vibrio cholerae* neuraminidase, 500 units/ml, BDH) for four hr at 37°C. Some papain digests were subjected to acid hydrolysis for either 40 or 60 min and 25 µl aliquots were analysed by descending paper chromatography using ethyl acetate-acetic acid-water (3:1:3, by vol) as solvent (Allen and Kent, 1968). After development for 48 hr the dried sheets were stained with thiobarbituric acid (Hunt, 1969) to test for the presence of interfering components of the type referred to by Aminoff (1961). N-acetylneuraminic acid was similarly treated and run as a marker on all chromatograms. Total protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951). Statistical calculations were performed either using a Mathatron 4280R calculator with preprinted programmes or an IBM 1800 digital computer.

Results

The concentrations of papain-solubilized protein, sialic acid, and total hexosamines per mg wet tissue are presented in the table. Since the protein/tissue wet weight ratios were constant and not significantly different in the two areas of mucosa, wet weight was used for reference in all estimations.

Hexosamines (µg of glucosamine-HCl/mg tissue

wet weight) showed higher values in transitional mucosa samples (1.90 ± 0.81 µg/mg) compared with the normal (1.38 ± 0.47 µg/mg). In spite of the wide range of values found the paired t test gave a value of 4.41, demonstrating a highly significant difference ($P < 0.001$). A similar significant difference ($P < 0.001$) was found for the sialic acid content of normal and transitional mucosa samples (table). Hydrolysis with neuraminidase gave much lower values for sialic acid. Thus, estimations made on a pool of eight samples gave sialic acid contents of 0.62 µg and 1.5 µg N-acetylneuraminic acid/mg tissue wet weight in normal and transitional mucosa respectively, compared with values of 1.0 µg and 2.0 µg/mg wet tissue respectively, when the same pool was analysed after acid hydrolysis. Thus, the enzyme released about 60% of the available sialic acid of normal mucosa but 75% of that available in transitional mucosa.

With transitional mucosa, paper chromatography showed only two components staining with thiobarbituric acid. The faster moving component had the same mobility as N-acetylneuraminic acid whilst the slower component only resolved poorly from it and probably represented an O-acetyl form although no reference marker was available. Normal mucosa gave only a single component with mobility similar to that of the slow component of transitional mucosa. In all cases the thiobarbituric-acid-positive spots were only just visible with digests of normal mucosa, whilst transitional mucosa gave strongly staining spots confirming that the sialic acid levels in transitional mucosa were considerably higher than in normal mucosa.

In order to exclude loss of sialopeptides on dialysis as the cause of the differences between normal and transitional mucosa, the dialysate was also analysed but no significant amounts of hexosamines and sialic acids were present. The dialysate was freeze dried, the residue redissolved with distilled water, and aliquots were taken for hexosamine and sialic

Type of Mucosa	Specimen No.															Mean $\pm$ STD	Paired t Test
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Protein (µg)/wet weight (mg)																	
Normal	1.21	1.44	3.85	2.82	3.12	3.26	2.88	3.31	3.16	2.59	2.92	1.03	5.31	4.28	3.53	3.08 ± 1.14	1.4733
Transitional	1.66	1.33	3.94	2.67	3.40	3.25	3.81	3.97	3.39	2.16	3.36	1.11	4.36	5.31	3.88		$P = 0.8$
Hexosamine (µg)/wet weight (mg)																	
Normal	0.86	1.08	1.73	1.46	1.23	1.59	1.36	2.05	2.22	1.03	1.16	0.63	1.03	1.14	2.07	1.38 ± 0.47	4.4106
Transitional	1.43	1.15	2.37	1.45	1.62	1.76	2.87	3.32	3.03	1.07	1.45	0.90	1.31	1.62	3.08	1.90 ± 0.81	$P < 0.001$
Sialic acid (µg)/wet weight (mg)																	
Normal	0.57	1.10	1.10	1.20	0.68	0.95	0.50	1.20	1.20	0.41	0.63	0.33	0.71	0.51	0.54	0.78 ± 0.32	5.3834
Transitional	1.00	1.14	1.98	1.83	1.26	1.23	1.94	2.35	1.77	0.58	1.05	0.62	0.81	1.05	1.00	1.31 ± 0.54	$P < 0.001$

Table Analysis of results for epithelial mucosubstances from human large intestine

acid estimation. No attempt was made to desalt the dialysate as it was felt that this could lead to loss of sialopeptide by absorption. However, sialic acid and hexosamine estimations were carried out both on the reconcentrated dialysates and also on a number of dilutions to compensate for the effects of salt concentration.

Discussion

Chemical analysis shows the presence of hexosamines and sialic acid in human large intestinal mucosa, in agreement with our previous histochemical and autoradiographic findings (Filipe, 1969; Filipe and Cooke, 1970; Filipe, 1971a and b) and other workers' biochemical studies in human (Sky-Peck, Lundgren, and Bornstein, 1966; Korhonen and Makela, 1971), pig (Werner, 1953) and sheep (Pasternak and Kent, 1958; Draper and Kent, 1963) colonic mucosa.

Transitional mucosa shows an absolute increase in both total hexosamines and sialic acid. The values are comparable to those obtained by Barker, Stacey, and Tipper (1959) in carcinoma of the human colon with a transitional/normal mucosa sialic acid ratio of 1.6 in this work compared with their ratio of 1.5 for 'invasive mucosa/normal mucosa'. Paper chromatography showed a change in mobility of sialic acid from transitional to normal mucosa consistent with the blocking of sialic acid by such mechanisms as O-acetylation (Andrews, Herring, and Kent, 1967) in normal mucosa.

The fact that there was no significant loss of either sialic acids or hexosamines into the dialysate is in favour of a real difference in hexosamine and sialic acid content between normal and transitional mucosa.

There have been many descriptions of tumour cells possessing features of foetal cells or capable of producing foetal proteins which can be detected in the sera of cancer patients (Gold and Freedman, 1965; Krupey *et al.*, 1968; Kleist and Burtin, 1969; Stonehill and Bendich, 1970; Lee, Rowley, and Mackay, 1970; Griffen and Meeker, 1972; Lo Gerfo, Herter, Barker, and Bennett, 1972). The production of these embryonic antigens in adult cells following malignant transformation may represent a loss of suppressor genes and a regression of the cell to a more embryonic state, a hypothesis supported by the changes described in the nucleic acid metabolism and proliferative capacity of colonic epithelium adjacent to carcinoma (Imondi, Balis, and Lipkin, 1969; Imondi, Lipkin, and Balis, 1970; Lipkin, 1970; Lipkin *et al.*, 1970; Troncale, Hertz, and Lipkin, 1971). Our results also suggest that the epithelial cells in the transitional mucosa

may be of immature type; they not only differ from the normal in content of hexosamine but show also a higher percentage of neuraminidase-sensitive sialic acids. A possible explanation is that in the transitional mucosa the cellular activation of blocking mechanisms such as O-acetylation (Andrews *et al.*, 1967) did not take place. On the other hand the overall picture of these cells producing mainly sialomucins rather than sulphated material is similar to the pattern found in the goblet cells in early foetal life (Lev, 1968). Available data do not permit us to conclude with certainty whether this is true or which factors are responsible for the regression to that embryonic type of activity (Gurdon, 1962; Winzler and Bekesi, 1967) which causes disruption of the normal pathway of glycoprotein synthesis and sulphation.

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