Regulation of the oncodevelopmental expression of type 1 chain ABH and Lewis^b blood group antigens in human colon by α-2-L-fucosylation

T F Ørntoft, P Greenwell, H Clausen, W M Watkins

Abstract
Blood group antigen expression in the distal human colon is related to the development of the organ and is modified by malignant transformation. To elucidate the biochemical basis for these changes, we have (a) analysed the activity of glycosyltransferases coded for by the H, Se, Le, X, and A genes, in tissue biopsy specimens from normal and malignant proximal and distal human colon; (b) characterised the glycosphingolipids expressed in the various regions of normal and malignant colon by immunostaining of high performance thin layer chromatography plates; and (c) located the antigens on tissue sections from the same subjects by immunohistochemistry. In both secretors and non-secretors we found a significantly higher activity of α-2-L-fucosyltransferases in carcinomatous rectal tissue than in tissue from normal subjects, whereas the other transferase activities studied showed no significant differences. The acceptor substrate specificity suggested that both the Se and the H gene dependent α-2-L-fucosyltransferases are increased in carcinomas. In non-malignant tissue the only enzyme which showed appreciably higher activity in caecum than in rectum was α-2-L-fucosyltransferase. Immunochemistry and immunohistochemistry showed α-2-L-fucosylated structures in normal caecum from secretors and in tumour tissue from both secretors and non-secretors. We conclude that the α-2-L-fucosyltransferases control the expression of ABH, and Lewis' structures in normal and malignant colon.

The antigens that constitute the ABO blood group system are oligosaccharides. Their biosynthesis is regulated by genes coding for specific glycosyltransferases that add monosaccharides to precursors in a sequential fashion (Fig 1). In this way the product of one transferase becomes the substrate of the next, and the loss of activity of one transferase leads to the disappearance of the antigen it would have formed and all subsequent antigens, which would have been synthesised with this structure or its derivatives, as acceptor substrates. This complicated inter-relation leads to a differential expression of blood group antigens in people with different genotypes. The role of the ABO, Se, H, X, and Le gene loci and their glycosyltransferase products in determining these antigens has been reviewed in detail elsewhere. These antigens are not only found in blood cells but are expressed in most epithelia and body fluids. In epithelia they have been shown to be differentiation depend-

ent, and several carbohydrate antigens have been suggested as tissue specific oncodevelopmental antigens.

A well examined epithelium is the colorectal mucosa. Both chemical analysis of carbohydrate structures and immunohistochemical analysis with monoclonal antibodies have shown that ABH related carbohydrate antigens carried by type 1 chain (Galβ1-3GlcNAc-R) core structures, as well as those carried by type 2 chain (Galβ1-4GlcNAc-R) core structures are expressed in the fetal colon and rectum, and that there is a gradual disappearance or appreciable reduction in expression of blood group antigens apart from Lewis' in the distal colon during fetal growth. In contrast, all type 1, and to a less extent some type 2, chain antigens are expressed in the proximal colon in adults. In carcinomas of the distal colon, the ABH, Le, Le, and Le antigens and extended, polyfucosylated, or sialylated derivatives of these antigens have been shown by immunohistochemical and immunohistochemical means to reappear or to be synthesised de novo in large amounts. This has made them suitable as tumour markers as more than two thirds of all colonic carcinomas are located in the distal colon.

The purpose of the present work was to examine the enzyme and structural basis for the differential expression of blood group carbohydrate antigens in the proximal and distal normal colon and in colorectal carcinomas. The enzyme findings have been correlated with immunohistochemical characterisation and immunohistochemical localisation of blood group antigens in tissues of the same subjects by the use of monoclonal antibodies with established carbohydrate specificities.

We report that control of the expression of type 1 chain, ABH, and Le structures in both normal and malignant colon is probably through α-2-L-fucosyltransferase, whereas type 2 chain structures are, in addition, subject to other regulatory mechanisms.

Methods

REAGENTS
GDP-L-[U-14C]-fucose (210–292 mCi/mm), UDP-N-acetyl-D-[1-14C]-galactosamine (54 mCi/mm), and UDP-D-[U-14C]-galactose (300 mCi/mm) were obtained from Amersham International Ltd UK. N-Acetyllactosamine (Galβ1-4GlcNAc), lacto-N-biose I (Galβ1-3GlcNAc), and 2'-fucosyllactose (FucO1-2Galβ1-4Glc) were kindly supplied by Dr A S R Donald, MRC Clinical Research Centre, Harrow, UK.

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Phenyl-β-D-galactoside was obtained from Koch-Light, Colnbrook; Triton X-100 was purchased from BDH Chemicals, UK. Diethyldiminoethyl cellulose (DE 81), and No 40 chromatography papers were obtained from Whatman, UK. Mouse monoclonal antibodies against blood group H and biotinylated swine antirabbit immunoglobulins were purchased from Dakopatts, Copenhagen, Denmark. Avidin-Biotin-Peroxidase complex was obtained from Vector Lab, Burlingame, California, USA. Mouse monoclonal antibodies against Le and Le\(^a\) antigens were kindly supplied by Dr L Messeter, the Bloodbank, Lund, Sweden. The specificity of anti-Le\(^a\) was tested against Le\(^a\), Le\(^b\), and Le\(^b\) glycolipids, that of anti-Le\(^b\) against Le\(^a\), Le\(^b\), H type 2 chain, Le\(^b\), and AL\(^b\) glycolipids (L Messeter, personal communication). Mouse monoclonal antibodies against Le\(^a\), clone AH\(_{4}\), IgG\(_{3}\), Le\(^b\), clone FH\(_{3}\), IgG\(_{3}\); A type 1 chain, clones AH21 (monofucosylated), IgG\(_{3}\); and HH\(_{3}\) (difucosylated), IgG\(_{2A}\); A type 2 chain, clones HH\(_{2}\) (mono- and difucosylated), IgG\(_{3}\); and HH\(_{4}\) (difucosylated), IgG\(_{3}\) were from the Biomembrane Institute, Seattle, Washington. 3-Amino-9-ethylcarbazole and Tris were purchased from Sigma Chemical Co, St Louis, USA.

**SPECIMENS**

Biopsy specimens of normal tissue were obtained from the rectum, sigmoid, left flexure, right flexure, and caecum from four A, 10 Le\(^a\) subjects, and one A, Le\(^a\)\(\cdots\) subjects. Tumour tissue was obtained from the rectum of 15 subjects. The tumours were graded as well differentiated two, moderate 10, and poor three; two were Dukes’ A, 10 were Dukes’ B, and three were Dukes’ C. Tumour donors were blood grouped as five A, Le\(^a\)\(\cdots\)\(\cdots\); one A, Le\(^a\)\(\cdots\)\(\cdots\); one A, Le\(^a\)\(\cdots\)\(\cdots\); five O, Le\(^a\)\(\cdots\)\(\cdots\); and three O, Le\(^a\)\(\cdots\)\(\cdots\). Le\(^a\)\(\cdots\)\(\cdots\) subjects were regarded as ABH secretors, Le\(^a\)\(\cdots\)\(\cdots\) subjects as ABH non-secretors. In the Le\(^a\)\(\cdots\)\(\cdots\) subject, saliva was used for determination of secretor state by haemagglutination inhibition.

**GLYCOSYLTRANSFERASE ASSAYS**

A modification of previously described\(^{22}\)\(^{23}\) assays was used. One gram of sample was solubilised in 1 ml 0.15 M NaCl, 2% Triton X-100 by sonication (Soniprobe, Dawes Instruments Ltd, UK) at 4°C, the resulting suspension centrifuged at 10 000 rpm for 30 min (Sorvall RC2-B. Rotor SS-34), and the supernatant used for the assays. For the assays of α-2-, α-3-, and α-4-L-fucosyltransferases, 20 µl of the enzyme source was added to GDP-L-[\(^{14}\)C]-fucose (0.56 nmol, 120 000 cpm), MnCl\(_2\) (1 µmol), acceptor (0.5 µmol), adenosine triphosphate (0.5 µmol), Triton X-100 500 µg, and Tris-HCl, pH 7.2 (5 µmol) in a total volume of 0.5 ml.
of 95 μL. The mixture was incubated at 37°C for 4 hours. When phenyl-β-D-galactoside was used as the acceptor substrate the mixture was spotted on Whatman No 40 paper and developed with ethylacetate/pyridine/water, 10:4:3 (by vol, solvent 1) for 4 hours. When N-acetyllactosamine, lacto-N-biose I or 2’-fucosyllactose were used as acceptors the incubation mixtures were chromatographed on Whatman No 40 paper in solvent 1 for 48 hours. The papers were dried and scanned for radioactivity in a Packard radiochromatogram scanner. The mobilities of the radioactive peaks were measured relative to known compounds and these areas were then cut out and counted by liquid scintillation spectrometry.

When α-2, α-3-, and α-4-L-fucosyltransferase activities occur together in an enzyme source, the substrate N-acetyllactosamine functions as an acceptor for both the α-2- and the α-3-transfereases, and lacto-N-biose I as an acceptor for both α-2- and α-4-transferases. Difucosyl products are seldom observed and the products of transfer to the 0-2 position of the terminal galactose residue (2’-fucosyllactosamine and 2’-fucosyllacto-N-biose I, \( R_{Lac} \) 1-0 and 0-94, respectively, in solvent 1) are separable from the product of transfer to the 0-3 (3-fucosyllactosamine, \( R_{Lac} \) 0-75 in solvent 1) or the 0-4 (4-fucosyllacto-N-biose I, \( R_{Lac} \) 0-68 in solvent 1) position of the subterminal N-acetyllactosamine unit. In contrast to these two acceptor substrates the phenyl-β-D-galactoside acceptor cannot be difucosylated.

α-3-N-acetyl-D-galactosaminyl transferase (UDP-N-acetyllactosamine:2-fucosylgalactoside α-3-N-acetyllactosaminyltransferase) activity was measured with incubation mixtures consisting of enzyme source 20 μL, UDP-N-acetyl-D-[\(^{14}C\)]-lactosamine (1-3 nmol, 12 000 cpm), adenosine triphosphate (0-5 μmol), MnCl\(_2\) (2-0 μmol), 2’-fucosyllactose (0-25 μmol), Triton X-100 (500 μg), and Na-cacodylate buffer pH 6-0 (5-0 μmol) in a total volume of 95 μL. The mixture was incubated for four hours at 37°C. The products were separated on Whatman DE 81 paper in propan-1-ol/ethylacetate/pyridine/water, 5:1:1:3 (by vol) for 16 hours. Duplicate determinations of glycosyltransferase activities varied by less than 10%.

**Glycolipid extraction**

A modification of the method described by Hakomori and Kannagi was used. The tissues were homogenised in isopropanol-hexane-water (55:25:20), sonicated, and centrifuged. The glycolipids in the supernatant were acetylated and subjected to florisil chromatography, deacetylated, and the neutral glycolipid fraction was isolated by diethyl-aminoethyl-Sephadex chromatography.

**Thin layer chromatography immunostaining**

A modification of the method described by Magnani et al \(^{22}\) was used. Briefly, approximately 20 μg of total neutral glycolipids were applied on a high performance thin layer chromatography (HPTLC) plate, and the plate was developed in a mixture of chloroform/methanol/water (50:40:10). Subsequently, the plate was blocked in 5% bovine serum albumin in phosphate buffered saline and incubated with monoclonal antibody, followed by incubation with rabbit antiserum IgG or IgM and \(^{125}\)I-protein A solution, successively. The dried plate was subjected to autoradiography for 18 to 24 hours by use of an intensifying screen and Kodak X-omat AR5 film.

**Immunohistochemistry**

Formalin fixed 4 μm sections were deparaffinised and rehydrated. Endogenous peroxidase was reacted with 0-03% hydrogen peroxide in methanol for 30 min. After washing, monoclonal antibodies were applied for 16 hours at 4°C. The sections were washed and sequentially incubated with rabbit antiserum immunoglobulins, biotinylated swine antirabbit immunoglobulins, and avidin-biotin-peroxidase complex, each step separated by washings in Tris-phosphate buffered saline (composition see above). Bound enzyme was visualised with 0-04% 3-amino-9-ethylcarbazole in N, N dimethylformamide 0-01% hydrogen peroxide.

**Results**

**Glycosyltransferase activity in normal and malignant rectum**

α-2-L-fucosyltransferases

α-2-L-fucosyltransferase activity was assayed with phenyl-β-D-galactoside, N-acetyllactosamine, and lacto-N-biose I as acceptor substrates. In Le(a-b+) subjects we found a significantly higher level of this transferase in tumour tissue compared with normal tissue with

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**Table 1**: α-2-L-fucosyltransferase activity in tumour tissue and normal tissue from rectum

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Tissue</th>
<th>Acceptor</th>
<th>Median</th>
<th>Lower and upper quartile</th>
<th>Lacto-N-biose I</th>
<th>Median</th>
<th>Lower and upper quartile</th>
<th>N-acetyllactosamine</th>
<th>Median</th>
<th>Lower and upper quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a-b+)</td>
<td>Normal</td>
<td>0-1</td>
<td>(0-1-0-3)</td>
<td>p&lt;0-02</td>
<td>3-3</td>
<td>(1-0-4-7)</td>
<td>p&lt;0-02</td>
<td>0-1</td>
<td>(0-1-0-1)</td>
<td></td>
</tr>
<tr>
<td>Le(a-b+)</td>
<td>Tumour</td>
<td>5-3</td>
<td>(2-6-7)</td>
<td>p&lt;0-01</td>
<td>3-3</td>
<td>(1-6-4)</td>
<td>p&lt;0-09</td>
<td>2-4</td>
<td>(1-6-7)</td>
<td></td>
</tr>
<tr>
<td>Le(a+b-)</td>
<td>Tumour</td>
<td>1-0</td>
<td>(0-8-1-3)</td>
<td>p&lt;0-04</td>
<td>1-3</td>
<td>(1-5)</td>
<td>p&lt;0-03</td>
<td>1-7</td>
<td>(1-6-1-8)</td>
<td></td>
</tr>
<tr>
<td>Le(a+b-)</td>
<td>Normal</td>
<td>3-0</td>
<td>(0-2-3)</td>
<td>p&lt;0-04</td>
<td>0-2</td>
<td>(0-1-0-2)</td>
<td>p&lt;0-05</td>
<td>0-2</td>
<td>(0-1-0-2)</td>
<td></td>
</tr>
</tbody>
</table>

NS = not significant at the p=0-05 level.

The activity was measured as pmol [\(^{14}C\)] labelled fucose incorporated into the acceptor/h/mg. Mann-Whitney two tailed test was used for statistical comparison.
**TABLE II**

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Tissue</th>
<th>No</th>
<th>Lower and upper quartile</th>
<th>Median</th>
<th>Lower and upper quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a+b-) and Tumour</td>
<td>14</td>
<td>15-5</td>
<td>(12-6-17-1)</td>
<td>8-4</td>
<td>(7-1-10-0)</td>
</tr>
<tr>
<td>Le(a+b-) and Normal</td>
<td>7</td>
<td>11-9</td>
<td>(7-2-14-9)</td>
<td>10-1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS = not significant at the p=0.05 level (Mann-Whitney two tailed test). α-4-L-fucosyltransferase was significantly lower than α-3-L-fucosyltransferase in tumour tissue (p<0.001). The activity was measured as pmol [3H]fucose incorporated into N-acetyllactosamine (α-3-L-fucosyltransferase) and lacto-N-biose I (α-4-L-fucosyltransferase)/h/mg.

![Graph showing ratios between fucosyltransferase activities in tumour tissue from rectum (10 subjects), and in different locations of normal colon (four subjects).](image)

**Figure 2:** Ratios between fucosyltransferase activities in tumour tissue from rectum (10 subjects), and in different locations of normal colon (four subjects). Circles indicate median, bars indicate lower and upper quartile. Open circles: α4/α2 ratio measured with lacto-N-biose I as acceptor. Closed circles: α3/α2 ratio measured with N-acetyllactosamine as acceptor.

α-3- and α-4-L-fucosyltransferases

No significant difference could be found between the activities in tumour tissue and normal tissue (Table II). α-3-L-fucosyltransferase was active in all samples examined, whereas α-4-L-fucosyltransferase activity was related to the Lewis type of the subject, being almost absent in Le(a+b-) subjects. In tumours, the α-3-L-fucosyltransferase was significantly higher than the α-4-L-fucosyltransferase, whereas both showed almost equal activity in normal tissue. α-2-L-fucosyltransferases compete with both the α-3- and α-4-L-fucosyltransferases for a mutual acceptor substrate (Fig 1). To evaluate alterations in this competitive action we calculated the ratios between the enzyme activities in each subject (Fig 2). The individual approach was necessary to eliminate interindividual variation. The α-4/α-2-L-fucosyltransferase ratio measured for both enzymes with lacto-N-biose I as substrate was significantly lower (p<0.005) in tumour tissue from rectum compared with normal rectal tissue (Fig 2). The α-3/α-2-L-fucosyltransferase ratio measured for both enzymes with N-acetyllactosamine as substrate was also significantly lower (p<0.005) in rectal tumour tissue than in normal rectal tissue.

α-3-N-acetyl-D-galactosaminyltransferase

This transferase was assayed at pH 6, which is the pH optimum for the A' transferase. Activity was only found in blood group A subjects (Table III). The activity in tumour tissue was lower (though not significant) than the activity in normal tissue. The individual ratios between the A' transferase and the fucosyltransferases with which it competes for acceptor substrates were not significantly altered.

GLYCOSYLTRANSFERASE ACTIVITY THROUGHOUT THE NORMAL COLON

The activity of the α-2-L-fucosyltransferase was higher in biopsy tissue from the caecum than from all other parts of the normal colon (Fig 3A). The high activity in caecum was more pronounced when lacto-N-biose I and phenyl-β-D-galactoside were used as acceptors than when N-acetyllactosamine was used. The α-3- and A' transferases (data for the A' transferase are not shown) were similar in activity throughout the colon. Because of this the individual α-4/α-2 ratio, and to a lesser extent the individual α-3/α-2 ratio, were much lower in caecum than in the rest of the colon (Fig 2). None of these regional

**TABLE III** α-3-N-acetyl-D-galactosaminyltransferase (A' transferase) activity in tumour tissue and normal tissue from rectum

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Tissue</th>
<th>A' transferase</th>
<th>A' transferase/α-4-L-fucosyltransferase*</th>
<th>A' transferase/α-3-L-fucosyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Lower and upper quartile</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower and upper quartile</td>
</tr>
<tr>
<td>0</td>
<td>Tumour</td>
<td>7</td>
<td>(1-0-3-3)</td>
<td>7-1</td>
</tr>
<tr>
<td>A'</td>
<td>Tumour</td>
<td>7</td>
<td>68-1 (48-9-86-5)</td>
<td>5-7</td>
</tr>
<tr>
<td>A'</td>
<td>Normal</td>
<td>5</td>
<td>103-2 (71-2-139-1)</td>
<td>8-4</td>
</tr>
</tbody>
</table>

*Only Lewis positive subjects were included. NS = not significant. Figures indicate pmol [3H]GlcNAc incorporated into 2'-fucosyllactose/h/mg. Mann-Whitney two tailed test was used for statistical comparison.
Regulation of the oncodevelopmental expression of type 1 chain ABH and Lewis' blood group antigens in human colon by α-2,6-fucosylation

Figure 3: (A) Glycosyltransferase activity throughout the normal colon of four secretors. Open triangles: α-2-FT activity with N-acetyllactosamine as acceptor. Open circles: α-2-FT activity with phenyl-p-D-galactoside as acceptor. Diamonds: α-2-FT activity with lacto-N-biose I as acceptor. Squares: α-4-FT activity with N-acetyllactosamine as acceptor. Symbols indicate median value. (B) Glycosyltransferase activity throughout the normal colon of three non-secretors. Symbols as in (A).

In carcinomas, the glycolipid profile was altered as compared with that of resected normal tissue (Fig 4). The tissue was subjected to thin-layer chromatography, deacetylated, and separated by DEAE-Sephadex chromatography. The total neutral glycosphingolipids obtained were separated according to sugar-chain length on high performance thin layer chromatography plates, and reacted with monoclonal antibodies against Lea, Leb, LeX, and LeY antigens. Binding of antibodies was visualised by 125I-protein A autoradiography.

IMMUNOHISTOCHEMICAL LOCATION OF BLOOD GROUP ANTIGENS

Normal specimens from resected normal tissue were stained in Le(a−b+) subjects by all the antibodies examined that were against type 1 chain H, Le', Lea, and A antigens, whereas type 2 chain specific antibodies against Lea, Leb, H, and A antigens reacted more weakly (Table IV). In Le(a+b−) subjects only the type 1 chain anti-Lea specific antibody showed strong staining in caecum (Table IV). In carcinomas from the...
rectum, however, all the antibodies gave positive staining patterns in Le(a−b+) subjects, and, remarkably, all but anti-H type 1, also in Le(a+b−) subjects (Table IV).

Discussion
The major difference observed in glycosyltransferase activity between normal rectal mucosa and rectosigmoidal carcinomas was the significantly increased activity of the α-2-L-fucosyltransferases in carcinomas from both secretors and non-secretors. This is in agreement with a previous report showing that, in comparison with normal colonic mucosa, the activity of the α-2-L-fucosyltransferase, as measured with phenyl-β-D-galactoside, was increased in colonic carcinomas of ABH secretors. The present study also showed that the α-2-L-fucosyltransferases were significantly higher in normal caecum than in normal rectum from secretors. All other transferases studied were of the same magnitude throughout the colon.

The biosynthesis of H, A and Le b blood group determinants from their common precursor largely depends on the activity of the α-2-L-fucosyltransferases (Fig 1). These antigens could be shown in rectal carcinomas and in normal caecum of secretors, but not in normal rectum. Therefore, we suggest that the α-2-L-fucosyltransferases control the expression of these blood group antigens in both normal and malignant human colon.

Non-secretors differed from secretors in the way that the α-2-L-fucosyltransferase activity was low in all regions of the normal colon, including caecum. It was remarkable, however, that rectal carcinomas from non-secretors showed a significant increase in α-2-L-fucosyltransferase activity with all three acceptor substrates, the main difference in enzyme activity between rectal carcinomas from secretors and non-secretors being higher activity with phenyl-β-D-galactoside and lacto-N-biose I in secretors. This interesting enzymatic finding in non-secretors was shown to lead to antigen synthesis, as Le a, A, and AL e antigens started to appear in rectal carcinomas from these subjects.

In colon mucosa there seem to be two forms of the α-2-L-fucosyltransferase, one that is Se gene dependent and has a preference for type 1 chain acceptors, and one, the H gene dependent, that is expressed independently of the Se gene and has a low activity with both type 1 and 2 chain acceptors. 29-32 In non-secretors no activity was present in caecum, and the activity observed in rectal carcinomas of non-secretors showed similar levels of activity with both N-acetyl-lactosamine and lacto-N-biose I as acceptor substrates. These data indicate that in non-secretors the H gene dependent α-2-L-fucosyltransferase is increased in colorectal carcinomas. In secretors the present data show higher levels of α-2-L-fucosyltransferase activity in normal caecum than in other regions of the colon with both type 1 and type 2 chain oligosaccharides and with phenyl-β-D-galactoside as acceptor. Increased activity with these acceptors was also seen in rectosigmoidal carcinomas compared with normal tissue from the same region (Table I). Phenyl-β-D-galactoside is an efficient acceptor for the α-2-L-fucosyltransferases and clearly distinguishes between secretors and non-secretors when used to assay material from submaxillary glands. 33 The high activity reported in secretors with this acceptor and with lacto-N-biose I, and the difference in activity between secretors and non-secretors in both carcinomas and normal caecum, indicate that in secretors it is the Se gene dependent transferase which is increased in caecum. In colorectal carcinomas from secretors an increase in activity of the Se gene dependent α-2-L-fucosyltransferase with an increase in both this transferase and the H gene dependent transferase could explain the present findings.

The α-2-L-fucosyltransferase competes with α-3- and α-4-L-fucosyltransferases for mutual acceptor substrates (Fig 1). If the α-2-L-fucosyltransferase acts first the H antigen and subsequently Le a and Le b antigens are formed, whereas if the α-3- and α-4-L-fucosyltransferases act first, Le a and Le b antigens are formed. The last two antibodies are poor substrates for the α-2-L-fucosyltransferase and cannot be fucosylated to form Le a and Le b antigens. To determine the prevailing pathway of type 1 and 2 chain biosynthesis (Fig 1), the individual ratios between α-4/(α-2- and α-3/(α-2-fucosyltransferases measured respectively with lacto-N-biose I and N-acetyl-lactosamine were calculated for each subject. Both ratios were significantly decreased in tissue from the caecum and rectal tumour tissue compared with normal rectal tissue. Thus in normal rectum type 1 chain Le a antigens are synthesised and no H, Le a, and A antigens are formed; a result which can be explained by the high α-4/(α-2- enzyme ratio. This ratio is lower in carcinomas and in normal caecum, and hence both Le a and H, Le a and A antigens are formed. Although type 2 chain blood group antigens may be synthesised by enzymes that also work on type

### Table IV

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Type 1 chain (Galβ3-4GlcNAc-R)</th>
<th>Type 2 chain (Galβ4-4GlcNAc-R)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Le</td>
<td>H</td>
</tr>
<tr>
<td>Secretors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal rectum</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Normal caecum</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Cancerous rectum</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Non-secretors (3 blood group 0, 1 blood group A):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal rectum</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Normal caecum</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Cancerous rectum</td>
<td>4</td>
<td>+</td>
</tr>
</tbody>
</table>

ABH secretor state determined on saliva specimens. Monoclonal antibodies were applied to tissue sections from secretors and visualised as described in methods. *Symbols in parenthesis=weakly and partially positive. †A antigens were only detected in the A subject.
1 chains, their biosynthesis is obviously regulated in a different way because the positional isomer of Leα, namely the Leβ antigen, is not detectable to any great degree in normal rectum. A lower α-3/α-2- ratio in carcinoma with only lead to formation of H type 2 chain antigens if the appropriate precursor structures are present. The lack of Le antigen in normal rectum indicates that this is not the case. Regulation of type 2 chain biosynthesis by enzymes that work on the synthesis of the type 2 chain core structure, as well as by enzymes that work on terminal fucosylation, thus seems probable.

The immunohistochemical characterisation of blood group antigen expression obtained by immunostaining of blood group active glycolipids verified the immunohistochemical detection of antigens in this and previous studies. As previously shown, only type 2 chain mucins are synthesised on extended chains; however, the mechanism leading to this difference between type 1 and 2 chains is at present unknown.

Reduction of α-3-N-acetyl-D-galactosaminyltransferase activity has previously been shown in intestinal carcinoma tissue. A reduction of this enzyme does not, however, seem to be important in the large bowel, as the present data confirm that carcinomas from A individuals can synthesise blood group A antigens when the α-2-fucosyltransferase level is increased.

The results suggest that the critical event required for blood group H, Leα, A, and B antigen biosynthesis, in both normal mucosa from the caecum and rectal carcinomas, is the expression of α-2-L-fucosyltransferase activity in this study was supported by the Danish Cancer Society.

References: