The Chemistry of Mucus, Part Two: The Composition of Glycoproteins from Gastrointestinal Carcinoma

The Scientific Basis of Medicine

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Black-and-white
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<**Opening titles**>

<**Schrager to camera** >

In the first part of my lecture, I described the isolation, composition and structural features of glycoproteins composing mucus. In the second part I report a comparative study of glycoproteins isolated from stomachs containing a carcinoma. 28 surgical specimens were investigated. Each specimen was divided into the carcinomatous area and the normal-looking mucosa and both parts were processed separately.

The methods used, the experimental details, are described in papers published from my laboratory.

<**Schrager narrates over graph, uses indicator; a chemical chart from the previous lecture is superimposed gradually over the graph**>

The extracts from the normal-looking mucosa and from the carcinoma of each specimen when eluted on gel chromatography were resolved into 3 fractions. A non-retarded fraction containing the bulk of the carbohydrate content and 2 retarded fractions consisting mainly of polypeptides. The glycoprotein isolated from the normal-looking mucosa contained virtually the same sugars as the glycoproteins isolated from gastric aspirates and gastric mucosa reported in the first part of this lecture. They consisted, the carbohydrate side chain consisted, of 4 galactose, 3 glucosamine and 1 galactosamine. Mild acid hydrolysis, pariate[?] oxidation and mass spectrometry provided data which showed that the carbohydrate side chain is composed of pairs, disaccharides. In a similar way as the carbohydrate side chain showed glycoproteins from normal gastric mucosa and gastric aspirates.

The pair nearest to the protein core consisted of N-acetylglucosamine and galactose; N-acetylglucosamine linking the side chain to threonine and serine. This was followed by 3 repeating disaccharide units consisting of glucosamine and galactose.

The general formula described in the first part of this lecture was also applicable to the carbohydrate side chains of the normal glycoproteins isolated from the normal-looking mucosa of each specimen. The n value was found to be 4. Superimposed on this basic structure were additional sugar residues, the blood group determines this. People with blood group specificity Lea have a fucose link to glucosamine, those with blood group H have an additional fucose link to galactose, an additional glucosamine was associated with blood group A and an additional galactose with blood group B.

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The glycoproteins, isolated from the malignant areas of each specimen, contained the same sugars as the normal glycoprotein […]

<**Schrager narrates over table showing information on malignant glycoproteins**>

[…] namely galactose, N-acetylglucosamine and N-acetylglucosamine. On the basis of the ratios of these sugars, the glycoproteins were divisible into groups, each group with a specific inner characteristic n value; it varied from n=3 to n=8. [?] degradation and mild acid hydrolysis as well as gastric chromatography and maspectrometry provided data for the sequence of the sugars.

< **Schrager narrates over chemical chart, uses indicator** >

The disaccharide units were the same as in the normal glycoprotein. The unit nearest to the core of the malignant glycoprotein was N-acetylglucosamine and galactose. The N-acetylglucosamine, as in the case of the normal glycoprotein, linking the carbohydrate side chain to the protein core. In addition to the basic structure some glycoproteins also contain sialic acid. Treatment of the sialitic proteins with neuraminidase or mild acid hydrolysis provided evidence that sialic acid was linked to the terminal galactose. In addition to a group of glycoproteins, which were sialated, 2 out of 20 glycoproteins contained also sulphate.

It is thus possible to divide, or, the malignant glycoproteins can be divided on the basis of their n value, on the basis of the sialic acid content and on the basis of the sulphate content.

< **Schrager narrates over table containing figures relating to amino acid abundances**>

The amino acid composition of the normal glycoproteins was similar to the amino acid composition of the glycoproteins isolated from gastric aspirates. That is to say, threonine and serine made up 40-50%, these together with proline, alanine and glycine 75-85%.

< **Schrager narrates over table listing malignant glycoproteins** >

The amino acid composition of the malignant glycoproteins differed from those of the normal. They were divisible into 2 groups on the basis of their threonine / serine ratio. The 5 amino acid analyses carried out showed that the threonine / serine ratio in 2 protein cores were similar to those of the normal, that is to say they were roughly 2:1. But 3 out of the 5 showed a normal ratio of 10, roughly, 5:1.

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<**Schrager to camera**>

The data provided by the 28 surgical specimens as well as the extracts from liver metastases and lymph node involvement showed a fundamental similarity between the normal and the malignant glycoproteins. They contained the same sugars, they also showed the same linkage namely N-acetylgalactosamine being linked to threonine and serine, […]

< **Schrager narrates over table listing carbohydrate abundances, as ratios** >

[…] they also showed the general formula of n/n-1, 1 was applicable both to the normal and malignant glycoproteins. The building block of both types of glycoproteins consisted of a disaccharide. But the results also reveal significant differences. These differences related to the n value, to the presence of sialic acid and also to the presence of sulphate.

<**Schrager to camera**>

The question arises whether these changes are degradative, fortuitous and not due to the presence or absence of special enzymes caused by genetic mutation. The results provide several conclusive pieces of evidence that the changes noticed in the malignant glycoproteins are essentially due to genetic mutation.

<**Schrager narrates over illustration of mucus cell which dissolves into earlier chemical chart**>

We have seen in the first part of this lecture the very complex and precise method of synthesis. It is as well to remember that a macromolecule consists of about 2500 amino acid residues and about 1500 carbohydrate side chains. In each carbohydrate side chain, the sugars of each carbohydrate side chain, were elaborately produced and linked by specialised enzymes in a definite and specific way. And also that the order and type of the sugars is specific for each glycoprotein. We have seen that some n values of the glycoproteins is above 4 which means that additional enzymes would have to be available to synthesise the nucleotype sugar and a special enzyme to transfer the sugar from the nucleotype sugar to the carbohydrate side chain.

< **Schrager narrates over table listing malignant glycoproteins** >

This would imply additional enzymes and would not support a suggestion that the changes are degradative. In a degradative change one would expect a more random and a smaller carbohydrate side chain, that is to say, n value being smaller than 4.

<**Schrager narrates over earlier chemical chart – a hand can be seen off-side moving the blocks of data around**>

The presence of sialic acid in some glycoproteins is again an indication that additional enzymes were present to link to manufacture sialic acid and to link it to the carbohydrate side chain.

A similar reasoning applies to the sulphated glycoproteins, as normal glycoproteins, the glycoproteins composing gastric mucus are neutral, contain neither sulphate nor sialic acid. The change of blood group specificity from blood group H to blood group A requires an additional sugar – it requires N-acetylgalactosamine linked to the non-reducing end of the carbohydrate side chain. The additional sugar would require an additional enzyme which again implies a genetic change providing additional enzymes in the assembly line of the carbohydrate side chain of the glycoproteins.

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< **Schrager to camera** >

The most significant finding of this investigation is the stability of the malignant glycoproteins. Glycoproteins isolated from different areas of the same cancer, the glycoproteins isolated from the gastric carcinoma and from the metastases in the liver and the metastases in the lymph nodes showed the same composition. They showed the same n value, the same content of sialic acid.

< **Schrager narrates over earlier illustration of mucus cell** >

It appears that once a genetic mutation has occurred that this mutation remains and the cells originating from the cell and its mutation, acquire the same properties.

<**Schrager to camera**>

The classification of malignant tumours is based on the histological criteria noticed on the mucosa of the gastrointestinal tract; abnormalities of the nucleus and structure of the epithelial cells and the tendency to invade surrounding tissue.

The results of this study provide a new approach to the classification of carcinomata of the gastrointestinal tract. It is possible to classify the malignant glycoprotein on the basis of its secretory product. It is possible to classify the malignant glycoprotein on the basis of its n value.

<**Schrager narrates over tables listing figures relating to malignant glycoproteins**>

Up to now we have found 5 different types of glycoproteins; the n value of each glycoprotein being constant within each group. A second classification could be

based on the threonine/serine ratio – 1 group having a ratio of 2:1 and a 2nd group having a threonine/serine ratio of 5:1.

<**Schrager narrates over earlier chemical chart**>

The glycoproteins could also be subdivided on the basis of the sialic acid content; some glycoproteins, some carcinomata contain sialic and neutral glycoproteins whereas others contain only neutral glycoproteins.

<**Schrager narrates over table containing figures relating to carbohydrate abundances as ratios**>

A 3rd classification could be based on the sulfation of the glycoproteins – a small number 2 out of 28 contain sulphate.

< **Schrager to camera**>

Whether the malignant glycoproteins possess specific antigenic properties which would distinguish them immunologically from one another and from the normal glycoproteins, this problem is now being investigated. Preliminary results are hopeful.

<**End credits**>