The Chemistry of Mucus, Part One: The Relation of Function to Structure

The Scientific Basis of Medicine

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University of London Audio-Visual Centre, 1975.
Made for British Postgraduate Medical Federation.

Film sequences of Cilia in action by courtesy of Lady Negus and the Royal College of Surgeons.
Produced by David R Clark.

Black-and-white
Duration: 00:33:35:09

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<**Opening titles**>

<**Schrager to camera** >

The gastrointestinal tract, the bronchial tree, the gallbladder, the cervical canal and the ducts of excretory glands are lined by a thin layer of mucus, secreted by cells situated in the mucosa of these organs. In some organs, for instance the stomach and the uterine cervix, every cell lining the mucosa is mucus secreting.

<**Schrager narrates over illustration of mucus cell which is shown at various points of close-up to follow his narration**>

The macromolecules composing mucus are synthesised in the Golgi apparatus. It is a cup-shaped structure, the base of which is situated just above the nucleus. Glucose is converted into the required sugars, galactose , N-acetylglucosamine and N-acetylgalactosamine, fucose and sialic acid – these sugars are linked to nucleotides, forming nucleotide sugars. The nucleotide sugars donate their sugars to the polysaccharides. The polysaccharide is then linked as side chains to the protein core, thus forming the glycoprotein. The glycoproteins are arranged in small bundles, encased in a membrane, pressed upwards and outwards and excreted into the lumen. One bundle is just seen here to emerge from the cell. Once in the lumen, the membranes rupture, the glycoproteins fuse to form a hydrogel. The hydrogel displays a unique combination of rheological properties […]

<**Schrager narrates over series of tables**>

[…] it shows the properties of a viscous fluid and of a gel-like semi-solid matrix. It shows cohesion, adhesion, viscoelasticity, a tendency to film formation; it retains static strain and a high ability of water retention. All mucus secretions share the rheological properties in a modified form. Some show the properties of a viscous solution while others those of an elastic solid. For example, saliva is essentially a viscous solution whereas the bronchial mucus displays properties of an easily distorted elastic solid; it shows cohesion, adhesion, viscoelasticity, elastic recoil, tensile strength and an ability to transmit static strain over long distances.

<**Schrager narrates over series of moving images of mucus movements and cilia**>

These properties facilitate the upward movement of a thin film of mucus, lining the bronchial tree, propelled by cilia; thus the trapped dust particles, bacteria and other debris are removed, keeping the airways clear.

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<**Schrager narrates over moving images of cervical mucus**>

There is evidence that the structure of mucus secreted by some organs is hormone dependent. Thus cervical mucus obtained during the proliferative phase of the menstrual cycle shows, under the influence of oestrogen, fine linear alignment in channel formation providing facilities for the directional movement of the spermatozoa. This is lost in the secretory cycle, in pregnancy, when progesterone predominates.

<**Schrager to camera**>

It is reasonable to suggest that the rheological properties of mucus are related to its function, but their precise role is ill-defined, not well understood and still open to conjecture. The rheology of mucus is primarily dependent on the composition in structure of the macromolecules composing mucus. The basic problem in the study of mucus is therefore […]

< **Schrager narrates over table** >

[…] to isolate the macromolecules composing mucus, to determine their composition and to relate function to structure. Thus any meaningful concept on the function of mucus has to be based on a large body of data, on the composition of the macromolecules composing mucus and on the forces which shape and mould these macromolecules into the 3-dimensional structure we recognise as mucus.

Until recently little precise information on the chemistry of the macromolecules composing mucus has been available.

< **Schrager to camera** >

Progress has been impeded by lack of reliable methods to isolate the macromolecules and to determine their composition and structure. The use of gas and gel liquid chromatography, adapted to the study of mucus, have provided means to study the macromolecules and to determine their composition. Data which have

accumulated during the last 10 years or so facilitated the analytical analysis of the composition of the macromolecules composing mucus, and provided a basis for a clearer understanding of the mucosal structure which may lead to a precise definition of their function.

Preliminary investigations were designed to find means of disrupting the semi-solid gel structure of mucus but maintaining its components intact as far as possible, and of selecting a fractionation procedure which would isolate the components of the liquefied mucus. The procedure selected involved 3 operations.

< **Schrager narrates over series of tables** >

1. The solubilisation of mucus, converting it into a form amenable to gel chromatography. This was achieved by treatment with hydrogen bond breaking reagents, of disulfide breaking reagents or incubation with pepsin at pH 1.5 or [?]ping pH 6.5. 2nd, the subsequent fractionation of the solubilised mucus by gel and ion exchange chromaphotography. 3rd, the determination of the carbohydrate into amino acid composition of the eluted fragments. Proteolysis did not significantly degrade the isolated lack of proteins as no differences were noticed in the composition of the glycoproteins isolated from mucus, solubilised with hydrogen bond breaking or disulfide bond breaking reagents or by proteolysis.

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<**Schrager narrates over graph**>

Gel chromatography resolves gastric, salivary, bronchial, biliary, small intestinal and seminal fluid into 2 or 3 fractions. A non-retarded fraction, showing a symmetrical peak, eluted at a void volume and containing the bulk of the carbohydrate content of the eluted substance in designated gastric, salivary, bronchial, biliary, small intestinal and seminal glycoproteins respectively. The 2nd and 3rd fractions consisted of polypeptides and the remaining carbohydrate content.

< **Schrager narrates over series of tables** >

I aim to determine 1. the composition of the carbohydrate and protein moieties[?] of the mucus glycoproteins; 2. the carbohydrate-protein linkage; 3. a detailed study of the composition and structural features of the carbohydrate components.

This work is based on the study of 300 individual gastric aspirates in extracts of gastric mucosa, 100 individual salivas, 150 bronchial aspirates, 150 individual bile samples, 100 individual seminal fluids, 30 extracts of small and 30 extracts of large intestinal mucosa. The investigation extended over a period of 12 years.

The amino acid analysis of all the mucus glycoproteins investigated is unusual but characteristic, providing criteria which distinguish the mucus glycoproteins from the serum glycoproteins and other polysaccharide protein complexes. Threonine and serine constitute 40-50% and these together with proline, alanine and glycine account for 75-85% of the total amino acid content. Only 10-20% of the macromolecule is protein, the rest is made up of the sugar moiety.

The threonine / serine ratio of the gastric, salivary, biliary, bronchial and seminal glycoproteins were roughly 2:1. The small and large intestinal glycoproteins were divisible into 2 groups on the basis of their threonine / serine ratio. In group 1 the ratio was found to be 2:1, whereas in group 2 the threonine / serine ratio was found to be 5:1. That is to say that for every serine residue there were 5 threonine residues. More detailed information is needed, especially information relating to the disposition of the 2 hydroxyamino acids in proline before an attempt could be made to construct a credible model of the protein core.

The large body of data provided by gas liquid chromatography show that all glycoproteins share the same sugars. They need galactose, N-acetylglucosamine and N-acetylgalactosamine. The analysis of this data also shows that it is possible to express the molar ratios of these 3 major sugars by a general formula: n/n-1 over 1. This formula is applicable to all glycoproteins no matter where they come from.

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< **Schrager narrates over chemical diagram** >

Mild acid hydrolysis and gastric gas chromatography, pariate[?] oxidation and mass spectrometry provided evidence that the sugars composing the side chain are arranged in pairs. The pair nearest to the protein core consists of N-acetylglucosamine and N-acetylgalactosamine linking the side chain to threonine or serine. This is followed by repeating units of of N-acetylglucosamine and galactose. Alkaline borohydrate treatment has provided 2 important pieces of evidence: 1. that galactosamine is the link between the carbohydrate side chain and the protein core; 2. that each carbohydrate side chain contains only 1 of N-acetylglucosamine

< **Schrager narrates over a series of tables** >

On the basis of these findings, the general formula n/n-1 over 1 expresses not only the ratios of the carbohydrate sugars, of the major sugars, but also of the average size of the side chain. It is also possible to express the number of the disaccharides – n representing the disaccharides of the individual glycoproteins. The value of n is specific for each glycoprotein. In gastric mucus, in gastric glycoprotein, the value is n; that is to say that glycoprotein composing gastric mucus consists of 4 galactose, 3 N-acetylglucosamine and 1 N-acetylgalactosamine. And n also expresses the number of disaccharides composing the side chain, namely the gastric glycoprotein consists of 4 repeating disaccharides.

In bronchial mucus and saliva the value of n is 4. In bile on the other hand the value of n varies between 4 and 10, that is to say, in some people the glycoprotein of bile would consist of 4 repeating units whereas in others it may have as many as 10 repeating units. In the small and large intestine, the value of n is 3. In the seminal fluid it is only 2.

<**Schrager, standing, narrates from in front of a wall chart**>

Mild acid hydrolysis, in fractionations of the fragments on bio-gel p2, pariate[?] oxidation, gastric chromatography and mass spectrometry show that the sugars on the carbohydrate side chains are arranged in pairs.

<**Schrager narrates over close-shot of wall chart as camera pans down**>

The pair nearest to the protein core consists of N-acetylglucosamine and galactose; N-acetylgalactosamine linking the carbohydrate side chain to threonine and serine. This is followed by 3 repeating units consisting of N-acetylglucosamine and galactose.

<**Schrager, standing, narrates from in front of a wall chart**>

The carbohydrate side chain of the mucus glycoprotein from the gastric mucosa illustrates the formula n/n-1 over 1 where n is 4. The carbohydrate side chain of the gastric glycoprotein consists of <*points to wall chart*> 4 galactose – 4=n, 3 glucosamines – glucosamine = n-1; and 1 galactosamine.

<**Schrager narrates as he moves the blocks of information on the wall chart around**>

The galactosamine links the carbohydrate side chain to threonine and serine. This is followed by pairs consisting of glucosamine and galactose. There is evidence that 1 glucosamine/galactose is linked to the galactosamine nearest to the protein core.

Superimposed on this general composition common to all glycoproteins, there are additional sugars which are associated with blood group specificity. People with blood group specificity Lea have a fucose link to glucosamine, people with blood group specificity H have an additional fucose link to galactose, people with blood group specificity A have a terminally situated galactosamine and people with blood group specificity B have an additional galactose.

The blood group determinants are not characteristic nor specific for the mucus glycoproteins as they are to be found in polysaccharide protein complexes of different composition and structure.

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Some mucus cells secrete in addition to galactose, glucosamine and galactosamine sialated and sulphated glycoproteins. In the bronchial sialated glycoprotein, the sialic acid is linked to the terminal galactose.

< **Schrager narrates over table** >

The most significant result to emerge from these studies is a unifying concept of the basic composition in structure of the isolated glycoproteins no matter where they come from. The results also provide evidence of group and organ specificity, the mucus glycoproteins of each organ showing a precise characteristic feature, differentiating it from the mucus glycoproteins of other organs. For example, the number of disaccharides composing the carbohydrate side chain is specific for each organ. The gastric glycoprotein contains 4 repeating disaccharides, whereas bile varies from 4-10, that is to say some people would have the bile glycoprotein of 4 repeating disaccharides, whereas in others it would be as many as 10 repeating disaccharides.

The composition of the glycoprotein in each group is constant. In the small intestine the repeating unit would be 3, whereas in seminal fluid only 2. Some glycoproteins are neutral, the gastric mucosa consists only of neutral glycoproteins. The bronchial mucosa consists of 3 types of glycoproteins – neutral, sulphated and sialated. The saliva contains only 2 – neutral and sulphated; whereas bile, similar to bronchial secretion contains 3 types – neutral, sulphated and sialated. It is reasonable to suggest that the precise structural differences of the glycoproteins of each organ affect and modify the rheological properties of the mucus of these organs.

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<**Schrager narrates over a series of diagrams**>

Mucus is a complex gel with several layers of organisation. The glycoprotein, the building block of mucus, is a sturdy covalently bound macromolecule. Its resistance to proteolysis would appear to provide a major advantage to the macromolecule which is synthesised and secreted into the proteolytic enzyme environment of the gastrointestinal tract. On the other hand the organisation of the glycoprotein into the 3-dimensional structure of the mucus gel is based on far less stable forces. It is maintained mainly by hydrogen bonding. Experimental data show that mucus disintegrates with a breaking of these bonds. The multiple interactions provided by a large number of individual polysaccharide side chains also have a stabilising effect on the gel. It forms a coherent mesh work, a kind of sponge which absorbs and immobilises enormous quantities of liquid. It consists almost entirely of water, yet none of it is free to flow through the gel. It organises water so it will have viscoelasticity, viscoelastic properties that enable it to code the mucosa.

Four samples of gastric mucus show that remarkable degree of hydration. It varies between 99.94% of water-99.85%. This waterproof barrier is extremely important as the fluid we drink and the food we eat is likely to form either a hyper or a hypotonic solution in the stomach […]

<**Schrager narrates over still image of gastric mucosa**>

[…] the former causing desiccation and the latter oedema of the exposed mucosal cells leading to their ultimate destruction. It thus appears to play a double role; it absorbs and immobilises large quantities of fluid and forms a barrier between the mucosal cells and the fluid which has not been absorbed. It modifies the fluid in intimate contact with the mucosa and safeguards a stable microenvironment for the mucosal cells.

The physiology of the stomach has built in elements which are hostile and destructive to gastric mucus.

<**Schrager narrates over diagram**>

The acid in pepsin components of the gastric secretion, the secretory product of the parietal chief cells produce environmental changes which are destructive to the mucus structure. The increased acid fragments and erodes the organisation of the mucus, it reduces the hydrogen bonding and frees the individual macromolecules. Once the macromolecule is detached from the main gel it becomes highly soluble because of its high carbohydrate content.

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< **Schrager narrates over close shots of a demonstration of some of the properties of mucus**>

The following pilot experiment illustrates the capacity of mucus as a barrier and the destructive effect of acid in pepsin on the integrity of mucus.

3 tubes were used – a piece of polythene meshing was placed between an adaptor in each tube. The adaptor fitted tightly into the top of the tube thus keeping the mesh in its place. A thin layer of mucus was spread on the mesh of each tube. A 10cm column of water was placed in 1 adaptor on top of the layer of mucus.

<**Schrager narrates over table listing the statistics of mucus as a barrier**>

It took 96 hours for the mucus to disintegrate and it remained intact for 48 hours. The addition of gastric secretion to the .5cm layer of mucus caused complete disintegration of the mucus layer after 5 ½ hours. In the 3rd tube sodium azide was added to the 10cm column of gastric secretion; the addition of sodium azide which inactivates pepsin retained the integrity of mucus for 10 hours. After 10 hours no difference was found between the tube with gastric secretion without and with sodium azide.

<**Schrager to camera**>

On the other hand, the physiology of the bronchial tree does not contain, as far as we know, elements which are hostile and destructive to the bronchial mucus. I suppose one could speak of good quality bronchial mucus by which we would mean an optimum degree of cohesion, high ability to transmit static strain over long distances. This would depend, it is suggested, on an optimum ratio of the neutral, sialated and sulphated glycoproteins, an optimum degree of hydration which in turn would depend on the intermolecular linkages and the lengths of the macromolecule and its branching. No detailed studies, as far as I know, have been done to elucidate these problems.

The data provided by this study show a fundamental similarity of all glycoproteins composing the mucus of different organs. And I believe that this fundamental similarity will account for the wide range of properties displayed by all mucus. The results also reveal qualitative and precise quantitative differences which, I believe, can account for the minor but crucial variations in the properties of each individual mucus secretion.

In order to break new ground, measurements of such unusual properties as elasticity, tensile strength and cohesion of mucus must be made, because these data will contain the information from which the detailed mechanism of interaction can be found. As always, more work needs to be done on the protein core because it is this which affects the distribution of the side chains and hence a confirmation of the individual glycoproteins.

After 12 years of work I can still only say that I am only at the beginning of understanding the chemistry of mucus.

<**End credits**>