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Progress report

The chemical composition and function of gastrointestinal mucus

Despite the long period in which gastroenterologists have been interested in mucus, few exact data and little precise information on this substance have been, until recently, available, due mainly to the lack of reliable procedures to isolate the components of mucus in a tolerably pure state, to determine their composition, and to study their structure.

The unique rheological properties of mucus, such as gelation, film formation, adhesiveness, and non-Newtonian viscosity, are intimately related to the chemical composition and structure of its components and the forces which mould them into the elaborately organized three-dimensional structure of mucus. It is also reasonable to suggest that the physical properties are intimately associated with the biological function of mucus. It follows, therefore, that a rational understanding of the function of mucus has to be based on a thorough knowledge of the composition and structure of this substance.

Electrophoresis and ion exchange methods used so successfully in the study of proteins were not helpful in separating and purifying the components of mucus. The biocolloids which constitute mucus do not lend themselves easily to electrophoresis. The concentration at which the initial band has to be applied to the paper is so high that the glycoprotein is itself a gel-like structure forming a visco-elastic network and therefore is not easily and uniformly manoeuvred by electrophoretic forces. Ion exchange resins are also unsuitable, as will be shown later.

The absence of exact quantitative methods to determine each of the carbohydrate components of mucus add to the difficulties. The commonly used methods involve non-specific colour reactions with various phenols in concentrated sulphuric acid but do not distinguish between the monosaccharides and are of limited use in estimating each sugar residue of the carbohydrate moiety of the components of gastric mucus.

Recent technical advances, especially the use of gas-liquid chromatography, to determine the sugar residues of complex mixtures, provide methods which, for the first time, have made possible the isolation of the components of mucus and their detailed study. Gas-liquid chromatography is based on the work of Sweeley[6], and the method and technical details have already been reported[5]. The exact quantitative estimation of micrograms of each carbohydrate residue of the mucus is well within the reach of this method, and has made possible the determination of each sugar and amino sugar of hydrolysates of single, small (5 ml) gastric aspirates.

A preliminary investigation was designed to find means of disrupting the structure of mucus but retaining its components intact, and of selecting a fractionation procedure which would isolate the liquefied mucus into its components. A suspension of mucus in a saturated solution of calcium chloride or 8 M urea was shown to liquify the mucus, and liquefied mucus, when eluted on a gel column, was resolved into well separated com-

ponents which thus became available for detailed study. Gel chromatography separates constituents of a solution according to their molecular size. Sephadex is the best known gel used. It is a dextran of bacterial origin crosslinked by a 1,3-glyceryl ether bridge giving a three-dimensional network. It is supplied in minute beads. When placed in a chromatographic column they act as a sieve for molecules of different sizes. Larger molecules that cannot enter the gel move through the column faster and thus become separated from the smaller ones. The smaller the molecules the longer they linger behind in the interior of the beads. The same is true for another hydrophilic preparation, a cross-linked polycrylamide (Bio-gel). The macromolecules, which are completely excluded from the interior of the beads, form the non-retarded fraction. Bio-gel P150 used in this investigation excluded all macromolecules above the molecular weight of 150,000.

Aspirated acidic gastric secretions contain gelatinous lumps which can be packed down by centrifugation. Occasionally a mucus layer is found floating on the surface. The deposit and floating material together form the so-called 'visible' mucus as distinct from the 'soluble' mucus contained in the supernatant[1]. A comparative study of these two phases of mucus was attempted in order (1) to isolate the components of the 'soluble' and 'visible' mucus; (2) to determine the carbohydrate and amino-acid composition of the isolated fragments and their partial characterization; and (3) to evaluate the relationship between these phases.

Fifty individual gastric aspirations were investigated. Each aspirate was centrifuged and the supernatant and deposit were studied separately. Preliminary investigations had shown that the floating layer and deposit had the same carbohydrate composition and they were therefore pooled. Adequate quantities were taken from the supernatant and deposit to determine the carbohydrate, sulphate, N-acetylneuraminic acid, and blood group specificity. The remaining material was eluted on Bio-gel P150, and the carbohydrate composition of each eluted fraction estimated.

The supernatant, 'soluble' mucus, and the deposit, 'visible' mucus, were resolved into well separated fractions when eluted on Bio-gel P150. The supernatant showed three peaks, namely, the non-retarded peak eluted at the void volume containing 69-95% of the glucosamine and 70-100% of the galactosamine put on the column, the second peak of pepsin, and the third peak containing polypeptides. The remaining carbohydrate was unevenly divided between the second and third peaks. The chromatographic profile of the deposit was similar to that of the supernatant except that it showed no pepsin fraction.

The non-retarded fraction of both types of mucus contained the same sugars, namely, galactose, fucose, glucosamine, galactosamine, and N-acetyl neuraminic acid. They also contained sulphate. As the non-retarded fraction contained by far the largest portion of the carbohydrate content and almost all the amino sugars, it is proposed to confine this investigation to the eluted non-retarded fraction, to be referred to in this paper as the 'gastric glycoprotein'.

Quantitative Relationship between the Carbohydrate Components of the Gastric Glycoprotein

The analytical data showed that the gastric glycoprotein has a basic structure common to all specimens investigated. It was found to be constant and showed the quantitative relationship: galactose: glucosamine: galactosamine as 4: 3: 1. The molar relationships were consistently molar ratios (Table I).

Carbohydrate Component	Blood Group Specificity			
	1 Non-secretor		2 H	
	Non-retarded Fraction	Deposit	Non-retarded Fraction	Deposit
D-Galactose	3·90 ± 0·173 (8)	4·14 ± 0·39 (5)	3·95 ± 0·080 (17)	3·95 ± 0·092 (16)
D-Glucosamine	3·00	3·00	3·00	3·00
D-Galactosamine	1·01 ± 0·26 (8)	0·90 ± 0·10 (5)	1·00 ± 0·045 (17)	1·00 ± 0·052 (16)
L-Fucose	1·89 ± 0·53 (8)	1·90 ± 0·28 (5)	2·60 ± 0·360 (17)	2·30 ± 0·660 (16)
Sulphate	1·50 ± 0·55 (8)	2·00 ± 0·81 (3)	1·37 ± 0·675 (5)	1·76 ± 0·780 (5)
Sialic acid	0·27 ± 0·15 (6)	2·14 ± 1·78 (3)	0·15 ± 0·070 (2)	0·27 ± 0·206 (4)

Table I *Carbohydrate components of gastric glycoprotein and blood group specificities*[1]

[1]The results are expressed as means ± S.D. with the number of aspirates investigated in parenthesis. Details of the relationships between the monosaccharides determined are given in the text.

Superimposed on the basic structure were terminal sugar residues which divided the glycoproteins investigated into groups, each group with a distinctive sugar residue and characteristic blood group specificity. All glycoproteins with the same terminal sugar residues showed the same blood group specificity. The characteristic feature of the glycoproteins of group 1 was their lower fucose content. An increase of fucose found in the glycoproteins of group 2 was associated with blood group specificity H. The addition of galactosamine or galactose to the glycoproteins of groups 3 and 4 respectively endowed these substances with blood group specificity A or B. An increase of galactose and glucosamine in the glycoproteins of group 5 was associated with A and B specificity. These findings are in agreement with the results of Morgan and Lloyd and their collaborators [4,3] in their study of the glycoproteins of pseudomucinous cysts.

The analytical data suggested that not all carbohydrate side chains of the glycoprotein terminated with the characteristic sugar determinant. The terminal galactosamine of group 3, the terminal galactose of group 4, and fucose of groups 1 and 2 showed restricted variations. This variation in the determining sugar residues was also found in glycoproteins of pseudomucinous cysts[3].

The division of gastric secretion with respect to blood group specificity corresponded to the division of the non-retarded fraction on the basis of the quantitative relationships between the carbohydrate components. It was thus possible to visualize and identify in chemical terms the blood group specificity of all the glycoproteins investigated.

Amino Acid Analysis

Amino acid analysis showed an unusual but characteristic composition. Threonine and serine constituted between 45 and 50% of the amino acid content. The ratio of threonine to serine was found to be approximately 2:1. Threonine, serine, proline, alanine, and glycine make up between 75 and 80% of the total amino acid content, which ranged between 14 and 19% of the glycoprotein (Table II).

Quantitative relationships were also found between the hydroxy amino acids and galactosamine (Table II). Threonine plus serine equalled galactosamine in all cases except in aspirations of group 2. The non-retarded fraction of group 2 (blood group specificity A, associated with an increase of galactosamine) and group 5 contained more galactosamine. The ratio of galactosamine to glucosamine varied in groups 3 and 5 between 3:1.2 and 3:2 instead of 3:1 as in groups 1, 3, and 4.

Blood Group Specificity					
3 A and H		*4 B and H*		*5 A, B, and H*	
Non-retarded Fraction	*Deposit*	*Non-retarded Fraction*	*Deposit*	*Non-retarded Fraction*	*Deposit*
3·94 ± 0·09 (16)	3·92 ± 0·11 (16)	4·69 ± 0·26 (9)	4·46 ± 0·41 (7)	4·48 ± 0·15 (3)	
3·00	3·00	3·00	3·00	3·00	
1·70 ± 0·22 (16)	1·25 ± 0·38 (16)	0·98 ± 0·35 (9)	1·00	1·55 ± 0·32 (3)	
2·80 ± 0·23 (16)	2·60 ± 0·11 (16)	2·70 ± 0·42 (9)	2·80 ± 0·43 (7)	2·30 ± 0·24 (3)	
1·35 ± 0·58 (5)	2·30 ± 1·11 (5)	1·20 ± 0·53 (4)	1·10 ± 0·73 (4)		
	0·20 ± 0·16 (4)	0·10 ± 0·05 (3)	0·16 ± 0·15 (4)		

Table I—*continued*

Ratio of carbohydrate components to N-acetylglucosamine (= 3·0).

Total Amino Acids	*Total Sugars*	*Percentage of Amino Acids in Glycoprotein*	*Amino Sugars*		*Threonine*	*Serine*	*Blood Group Specificity*
			Glucosamine	*Galactosamine*			
0·631	3·696	14·57	1·00	0·336	0·212	0·110	H
0·807	4·307	18·00	0·98	0·300	0·212	0·109	H
0·482	2·774	15·00	0·780	0·258	0·182	0·087	H
0·263	1·320	17·00	0·365	0·120	0·066	0·033	H
1·275	5·695	19·00	1·560	0·527	0·385	0·202	H
1·716	9·350	15·50	2·400	0·800	0·562	0·286	H
0·988	8·470	10·45	1·54	0·770	0·358	0·179	A & H
1·010	7·786	14·00	1·24	0·770	0·258	0·129	A & H
1·444	7·200	18·00	2·10	1·030	0·518	0·263	A & H
0·375	2·178	18·00	0·395	0·176	0·079	0·047	A & H

Table II *Total amino acid, carbohydrate, and threonine and serine content of the eluted non-retarded fraction of the visible mucus of 10 gastric aspirations*[1]

[1]Quantitative relationships are to be found between the total amino acids and carbohydrates. Threonine: serine is 2:1.
The percentage of the protein content varies between 12 and 20%.
Galactosamine equals approximately threonine + serine (except in glycoprotein with blood group specificity A + H). All specimens are expressed in mmol/litre.

Sulphate

The sulphate content was variable. The sulphate/glucosamine ratio varied between 1/10 and 1/1. Its exact position and linkage have yet to be established.

Ultracentrifugation

Ultracentrifugation studies revealed a uniform peak suggesting homogeneity with reference to this method.

Light Scattering

Light scattering measurements suggested that the macromolecules composing the non-retarded fractions are rod-shaped (rigid), and the large ones are of the order of 10,000 Å end to end. The approximate molecular weight is $2{\cdot}04\ 10^6 \pm 7\%$.

Distribution of Glycoprotein between the Supernatant and Deposit

The distribution of glycoprotein appears to be determined by the acidity of

the secretion and the time interval between the aspiration, centrifugation, and separation of the supernatant from the deposit. The deposit of freshly aspirated gastric secretion of low acidity contained up to 80% of the total carbohydrate content. Gastric aspirations at neutral or near neutral *p*H are often found to be in a semi-gel form and no supernatant was obtained after centrifugation.

The results of this investigation demonstrate that the non-retarded fractions of the eluted supernatant and deposit of the centrifuged gastric secretions have the same carbohydrate and amino-acid composition and similar structural features. The analytical data on the carbohydrate and amino-acid composition and the quantitative relationship between galactosamine and the two hydroxy amino acids revealed no significant differences between the glycoproteins composing the two phases of gastric mucus, and also suggest that the glycoprotein composing the non-retarded fraction is the principal glycoprotein secreted by the gastric mucosa as it contained 70-100% of the galactosamine put on the column.

These results can be reconciled with the findings of other workers in this field which were obtained mainly by electrophoresis and ion exchange chromatography, methods which have been used almost exclusively in the investigation of the carbohydrate-containing fraction of gastric secretion. Previous studies, confirmed by this investigation, showed that the gastric glycoprotein is polydisperse with reference to sulphate and sialic acid[5]. Variation in the number of these end groups amongst the macromolecules endow the gastric glycoprotein with varying degrees of electrophoretic mobility. It is suggested that the multiple bands found by electrophoresis and the multiple fractions eluted in ion exchange chromatography arise from variations in the two charged end groups attached to a common macromolecular component rather than from major differences in its backbone structure.

This study has shown that the carbohydrate and amino-acid composition provides distinct and distinguishing features which facilitate the identification of the gastric glycoprotein, namely, the quantitative relationships between the carbohydrate components; the presence of galactosamine and the absence of mannose; blood group specificity; a characteristic amino-acid composition; the quantitative relationships between galactosamine, threonine, and serine; and the high carbohydrate content of the glycoprotein (86-89%).

The data on the distribution of the glycoprotein between the supernatant and the deposit shed light on the two phases of mucus. Gastric aspirations at neutral or near neutral *p*H were often found to be in a semi-gel form and no supernatant was obtained after centrifugation. The increase in the glycoprotein content of the supernatant and a corresponding fall in the 'visible' mucus appears to be related to the acidity of the secretion. The lower the *p*H, the higher the concentration of the glycoprotein in the supernatant. It was also shown that the glycoprotein becomes more soluble with time between aspiration and centrifugation. Simultaneously with an increase in the soluble glycoprotein there was noticed an increase in free sialic acid.

These findings suggest that the freshly secreted product of the mucus cell is in a gelatinous form. The secretions of the neighbouring mucus cells fuse and form a continuous layer, a protective covering for the mucosa, and the mucus cell may therefore be thought of as an organ producing a substance with a definite physiological function, namely, that of protecting and stabilizing the micro-environment of the mucosa.

In 1963 Bennett[2] suggested the term 'glycocalyx' as a broad term which includes all polysaccharide-containing structures on the external surfaces of cells. Evidence is steadily accumulating for a carbohydrate-containing component on or near the periphery of all cells forming the outer barrier between the environment and the cell. This surface coat may play a decisive role in controlling the external environment of each cell, and the mucus cell may

well be a specialized cell manufacturing a substance which forms the outer coating for the gastrointestinal tract. The glycocalyx-like substance secreted by the mucus cell shares with the red cell glycocalyx some structural features, hence the same blood group specificity.

The macromolecule, the building unit of the macro system recognizable as mucus, has a basic stable composition and structure and is not affected by the environmental changes encountered in the gastric secretion, as all its molecules are linked by primary valency bonds. Previous investigations have shown that it resists papain. It does not appear to be affected by pepsin, as no differences could be found in its composition and structure and molecular weight by ultracentrifugation between glycoproteins of acid secretion and glycoproteins obtained from gastric secretion with a *p*H near neutral.

The structure of mucus is maintained by far less stable forces. The architecture and configuration at the molecular level of the structure of mucus is maintained by electrostatic forces supplied by the charged end groups, sulphate and carboxyl groups, and hydrogen bonding. The multiple interactions provided by the large number of individual polysaccharide side chains also have a stabilizing effect on this network.

Solvent will flow with some difficulty through the fine meshwork, and molecules the size of pepsin will be retarded. This elaborately organized structure acts as a filter, regulating the passage of molecules according to size. The large number of charged end groups endow it with properties of a micro ion exchanger. By virtue of its filtration and selective binding, the mucus structure modifies the fluid in intimate contact with the mucosa.

Rigid crosslinking is prevented by the bulky carbohydrate side chains and regional hydrophobic properties contributed by some of the side chains of the protein core and fucose. The balance of forces provides a flexible protective covering, so safeguarding a stable micro environment for the underlying mucosal cells.

The effectiveness of the forces which maintain the integrity of mucus is lowered with increasing ionic strength and the lowering of the *p*H. The increased electrolytes screen the charged end groups, thus reducing greatly their internal binding force and added H ion upsets the balance by reducing the negative charge.

The acid pepsin component of the gastric secretion, the secretory product of the parietal and chief cells, produces environmental changes—increased acidity and ion concentrations and marked increase of diluting fluid—which are destructive to the mucus structure. The increased acid fragments and erodes the organization of mucus. It suppresses the electrostatic forces, reduces the hydrogen bonding, cleaves off the sialic acid, and frees the individual macromolecules. Once the macromolecule is detached from the main gel, it becomes highly soluble because of its high carbohydrate content (86-89%), hence the increased glycoprotein of the supernatant. The marked increase of fluid also has a destructive effect. For any system capable of gel formation there will be a limit to dilution when gel formation becomes impossible. If this condition occurs bringing about demixture, flocculation is observed instead of gel formation.

As long as the production of mucus does not lag behind the destruction, the protective and destructive forces are in dynamic equilibrium. The capacity for complete recovery and repair, even while the insult continues, helps to maintain the efficiency of the protective layer of mucus. Its efficiency also depends on its structural quality. The stability of the complex would increase with an increase in the number of possible sites for electrostatic interaction of its components, that is, with a larger number of sulphate and carboxyl groups.

The accumulated data form a broad basis for a meaningful concept of the composition and structure of its building unit, the gastric glycoprotein.

This concept facilitates a clearer definition of the problems to be studied, which relate to the organization of mucus and the forces which maintain it. The physical properties of mucus depend not only on the actual building unit but even more on the overall molecular architecture of the complex. The organization of mucus can maintain itself by continual renewal, depending on the quantity and quality of the secreted mucus. The micro anatomy of the structure of mucus, the variation in the structure, and the physiological limits of these variations are fundamental questions which have to be answered before a better understanding of the function of mucus can be gained.

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