The Carbohydrate Complexes of Bronchial Secretion

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The mammalian respiratory tract maintains resting levels of secretion which are subject to the external environment and also to nervous stimuli from within (Florey, Carleton & Wells, 1932). The secretion from the bronchial mucosa of the healthy human subject is, however, rarely available for investigation as normally it is swallowed together with the secretions of the upper respiratory tract and bucco-pharynx. In contrast, sputum, which is the pathological counterpart of bronchial secretion, is relatively easy to obtain, particularly during winter, in sufficient quantities for study. None the less, no substantial work on the composition of the mucous ground substance of sputum has been done since the original work of Müller (1896).

In this Laboratory, interest in the mucin of sputum was stimulated by the work of Himmelweit (1948), who found the mucin to contain a carbohydrate complex (F. Himmelweit, personal communication) which was an inhibitor of influenza-virus haemagglutination; his work has been confirmed by Curtain, Marmion & Pye (1953). The present paper describes the isolation, purification and properties of the carbohydrates contained in pooled sputa. A comparison of the properties of one of these carbohydrates with those of a similar mucoprotein occurring in urine is also made.

MATERIALS AND METHODS

Sputum. Sputum samples were collected from patients who were suffering from long-standing inflammatory conditions of the lung, such as chronic bronchitis. The samples, which were stored in a refrigerator at 0–4°, were sorted each week and only those which were either 'mucoid' or faintly 'muco-purulent' were retained. The terms 'mucoid' and 'muco-purulent' are clinical assessments of sputum based on its gross appearance (Chamberlain, 1957). 'Mucoid' sputum is either colourless or white and has a gel-like consistency. 'Muco-purulent' sputum has the same consistency as 'mucoid' sputum but has a yellow or green tinge due to the admixture of pus, the colour of the sputum depending largely on the amount of pus present. The selected samples were pooled, washed with water and dried with acetone. The resulting grey-white powder, termed acetone-dried sputum, was used for this investigation. A total of 1216 specimens was collected, of which 385 were selected for use; the volume of sputum obtained from the chosen samples was 1.925 l., which in turn yielded 28.6 g. of acetone-dried sputum.

Urine. Urine from normal males was collected in 2.5 l.

bottles. Chloroform (10 ml./l.) was added and the bottles were stored in a refrigerator at 0–4°.

Total nitrogen. This was estimated by an adaptation of the Markham (1942) steam-distillation technique, followed by colorimetric determination of the nitrogen as ammonia by the nesslerization procedure of Koch & McMeekin (1924). Combustion of the samples was carried out with the copper-selenium reagent of Campbell & Hanna (1937). A.R. $(\text{NH}_4)_2\text{SO}_4$ and thrice-recrystallized glucosamine hydrochloride were used as standards.

Phosphorus. Organic and inorganic phosphorus were determined by the modified Fiske & Subbarow (1925) method described by Kabat & Mayer (1948).

Reducing sugars. Estimation of reducing sugars was carried out by the method of Somogyi (1945) and is expressed in terms of glucose.

Amino sugars. The procedure devised by Schloss (1951), which is based on the method of Elson & Morgan (1933), was used to estimate amino sugars in terms of glucosamine. Schloss (1951) recommended that the complex which is formed when a solution of glucosamine is treated with acetylacetone and coupled with *p*-dimethylaminobenzaldehyde, in the manner he describes, should stand at 30° for 24 hr. This incubation allows the absorption spectrum of the glucosamine complex, which initially has a maximum at 530 m μ , to change to a more stable pattern with a maximum at 512 m μ . It was found, however, that the glucosamine complex attained a stable absorption spectrum with a maximum at 512 m μ if the solution stood at 22.5° for 24 hr.

Partition chromatography. The ascending-chromatographic technique described by Williams & Kirby (1948) and Bray, Thorpe & White (1950), with Whatman no. 4 paper, was used for sugars. The hydrolysates of the mucopolysaccharides and of the mucoproteins were prepared for chromatography by the following modification of the method of Malpress & Morrison (1949). The hydrolysates, which were dried *in vacuo* over H_2SO_4 and NaOH, were extracted at 60° with 0.4 ml. amounts of dry pyridine. The resulting solutions were filtered by suction through Pasteur pipettes, the ends of which had been constricted and plugged with filter-paper pulp, and the filtrates were again dried *in vacuo*. The process was repeated several times to ensure maximum de-ionization of the hydrolysates. The solvent systems used for irrigation were as follows: butanol-acetic acid-water (40:10:50, by vol.); pyridine-butanol-benzene-water (30:50:10:30, by vol.); collidine-water; phenol-water. Development was carried out with aniline hydrogen phthalate and the silver nitrate reagent of Trevelyan, Procter & Harrison (1950). Two-dimensional chromatography was used to identify the amino acids in the mucopolysaccharides, by the method of Consden, Gordon & Martin (1944). The papers were irrigated with the butanol-acetic acid-water and the pyridine-butanol-benzene-water mixtures described above. Ninhydrin was used to show the amino acid spots.

pH. This was determined with a Cambridge pH meter and a saturated KCl soln.—calomel/glass electrode system.

Viscosity. The modification of the Ostwald viscometer described by Cannon & Fenske (1938) was used to determine the viscosity of 0.2% solutions of the mucopolysaccharides and mucoproteins in 0.85% NaCl soln. and in water. The measurements were carried out at $25 \pm 0.01^\circ$. The results were expressed as the relative kinematic viscosity (ν) relative to that of the solvent in which the carbohydrates were dissolved.

Colorimetric and spectrophotometric measurements. These measurements were carried out on a Hilger Spekker photoelectric absorptiometer and on a Beckman quartz spectrophotometer.

Electrophoresis. A Perkin-Elmer Tiselius apparatus was used.

Urinary mucoprotein. This complex was isolated from the collected urine by a modification of the method of Tamm & Horsfall (1952). The material when freshly prepared was completely soluble in 0.025M- Na_2HPO_4 - NaH_2PO_4 buffer, pH 6.8, but after a lapse of 3 months it was found to be only partly soluble in this buffer. The mucoprotein was found to be electrophoretically homogeneous.

EXPERIMENTAL AND RESULTS

Isolation of the mucopolysaccharides and the mucoprotein

Digestion with proteolytic enzymes, originally employed by F. Himmelweit (personal communication) to extract the virus haemagglutinating inhibitory complex from acetone-dried sputum, was used here as the first stage of the isolation procedure. Pepsin was adopted as the enzyme of choice as it resolved the acetone-dried sputum into a supernatant, which contained the mucopolysaccharides, and an insoluble component which was largely composed of the mucoprotein. Incubation with the enzyme was carried out for 40 hr. It was found unnecessary to prolong the time of incubation to the periods of days and weeks that were employed by Kabat, Bendich, Bezer & Beiser

(1947) to isolate blood-group mucopolysaccharides from sources such as saliva.

Several methods of purifying the mucopolysaccharides obtained from peptic digestion of acetone-dried sputum were tried; fractionation with 90% (w/v) phenol-water by the technique of Morgan & King (1943) gave the most satisfactory results. The details of the procedure used for isolating the mucopolysaccharides and the mucoprotein are given below.

Enzymic digestion. Acetone-dried sputum (5 g.) was dissolved in 1 l. of water at 0° and the solution was adjusted to pH 2.3 with 0.1N-HCl. Armour crystalline pepsin (50 mg.) was added and the mixture was incubated at 37° for 40 hr. under a layer of toluene. At the end of this time, the mixture was cooled and centrifuged at 0° to remove the insoluble fraction and the toluene.

Isolation of the mucoprotein. The insoluble fraction was washed repeatedly with water acidified with HCl to pH 2.3. It was then washed with ethanol and ether and dried *in vacuo* over P_2O_5 ; 620 mg. of grey-white powder was obtained. The bulk of this material was dissolved in water, and dialysed at 0° against water for 14 days and centrifuged at 10 000 g to remove insoluble residue. The mucoprotein contained in the resulting solution was dried from the frozen state. A voluminous white powder (350 mg.) was obtained.

Isolation of the mucopolysaccharides. The supernatant liquid from the peptic digestion was made 0.1M with respect to sodium acetate, and adjusted to pH 8.0 with 0.1N-NaOH and the mucopolysaccharides were precipitated with 2 vol. of ethanol at 0° , precautions being taken to obviate a rise in temperature due to the mixing of ethanol and water. The precipitate was redissolved in water and dialysed against water at 0° to reduce the large inorganic phosphorus content (Table 1), which would have interfered with the subsequent phenol

Table 1. *Composition of the mucopolysaccharides and the mucoprotein of sputum and of these complexes during the various stages of their isolation from acetone-dried sputum*

Analyses of the complexes were carried out on samples which were dried at 56° for 1 hr. The analytical results are expressed as a percentage of this dry weight. For methods of analysis, see text.

Preparation	Yield (%) [*]	Total N (%)	Amino sugars (%)	Reducing sugars (%)	Total P (%)	Inorganic P (%)	Organic P (%)
Acetone-dried sputum	—	10.4	10.8	18.7	0.62	—	—
Complex from peptic digestion insoluble at pH 2.3 (mucoprotein)	12	8.7	14.5	26.2	0.78	0.02	0.76
Complex from peptic digestion soluble at pH 2.3 (mucopolysaccharides)	25	5.9	20.4	38.3	1.13	1.06	0.07
Mucopolysaccharides after dialysis	22	6.1	22.5	45.8	0.33	0.25	0.08
Phenol-insoluble mucopolysaccharide	10	5.2	28.4	57.6	0.12	0.04	0.08
Phenol-soluble mucopolysaccharide	2	5.7	29.9	57.5	0.12	0.04	0.08
Mucoprotein	7	8.7	14.3	26.3	0.75	0.01	0.74

^{*} The percentage yield was computed on a w/w basis with the parent acetone-dried sputum.

fractionation. The mucopolysaccharides were then recovered by ethanol precipitation as described above, and the precipitate was dried *in vacuo* over P_2O_5 . This product (0.85 g.) was fractionated with 90% (w/v) phenol-water in a manner similar to that described by Morgan & King (1943) and Annison & Morgan (1952*a, b*). The procedure yielded a phenol-insoluble mucopolysaccharide, and also a phenol-soluble mucopolysaccharide which was isolated from the 90% phenol-water mixture by precipitation with ethanol at 10% of ethanol by vol. These two complexes were dissolved in water, and dialysed for 7 days against water at 0° and dried from the frozen state. There was obtained 394 mg. of the phenol-insoluble mucopolysaccharide and 84 mg. of the phenol-soluble mucopolysaccharide. Table 1 gives the results of the isolation of the mucopolysaccharides and the mucoprotein.

Properties of the mucopolysaccharides and the mucoprotein

Physical properties. The relative viscosity (ν) of a 0.2% solution of the phenol-soluble mucopolysaccharide in aq. 0.85% NaCl soln. at 25° was found to be 1.32. The relative viscosity of a 0.2% solution of the phenol-insoluble mucopolysaccharide, measured under the same conditions, was 1.51, and that of an 0.2% solution of the mucoprotein in aq. 0.85% NaCl soln. was 1.61.

Electrophoresis of the phenol-insoluble mucopolysaccharide and of the mucoprotein was carried

out in a 0.02M- Na_2HPO_4 - NaH_2PO_4 buffer, pH 7.4, containing 0.15M-NaCl at a potential difference of 105v and a current of 20 mA. There was not enough of the phenol-soluble mucopolysaccharide for this determination. The ascending electrophoretic pattern of the phenol-insoluble mucopolysaccharide, photographed after 3 hr. (Fig. 1*a*), shows clearly that two components were present in a proportion of approximately 2:3. In contrast, the ascending pattern of the mucoprotein (Fig. 1*b*) recorded at 75 min. shows a single sharp boundary, indicating that the material is essentially electrophoretically homogeneous. Similar results were obtained at 2 hr. and at 3 hr. Both the electrophoretic pattern of the phenol-insoluble mucopolysaccharide and that of the mucoprotein showed a higher δ -boundary peak than might have been expected. This may represent in each case a slow-moving or stationary component of electrically near-neutral character at this pH. After one of the electrophoretic runs on the mucoprotein, this component was removed from the cell and its ultraviolet-absorption spectrum curve determined. This was found to be characteristic of a complex of protein nature and to show a peak at 277 $m\mu$.

Chemical properties. Aqueous solutions of both the mucopolysaccharides and the mucoprotein were of neutral pH. Lowering the pH of solutions of the mucoprotein below 3.3 with 0.1N-HCl caused the complex to precipitate; addition of N-HCl to a final concentration of 0.5N-HCl caused it to redissolve. Neither the phenol-insoluble mucopolysaccharide nor the phenol-soluble mucopolysaccharide was precipitated from aqueous solutions by lowering the pH, or by boiling, or by the addition of equal volumes of reagents that normally precipitate proteins.

The presence of alkali-labile *N*-acetylhexosamine linkages was demonstrated in all three preparations by the method of Morgan & Elson (1934). The red solutions obtained from the two mucopolysaccharides were examined spectrophotometrically, and two absorption maxima at 548 and 588 $m\mu$ were found. These observations are in accord with those reported by Aminoff, Morgan & Watkins (1952) for the action of alkali on specific blood-group mucopolysaccharides.

When the phenol-insoluble and the phenol-soluble mucopolysaccharides were hydrolysed with 0.5N-HCl in sealed tubes in boiling water complete liberation of hexosamines and reducing sugars occurred in about 2 hr. There was no detectable SO_4^{2-} ions either in the hydrolysates of the mucopolysaccharides or in the hydrolysate of the mucoprotein, which showed that sulphuric acid esters were absent from all three substances.

The hexosamines of the hydrolysed mucopolysaccharides and mucoprotein were allowed to

Fig. 1. (a) Ascending electrophoretic pattern of the phenol-insoluble mucopolysaccharide, scanned at 180 min., showing the two components of this complex. (b) Ascending electrophoretic pattern of the sputum mucoprotein, scanned at 75 min., showing the single sharply defined boundary. Both the electrophoretic runs were carried out in 0.02M- Na_2HPO_4 - NaH_2PO_4 buffer, pH 7.4, containing 0.15M-NaCl, at 100v and 20 mA. The concentration of the phenol-insoluble mucopolysaccharide was 2% (w/v) and that of the mucoprotein was 0.7%. The arrows indicate the direction of migration of the boundaries.

react for 20 min. at 100° with acetylacetone in a solution of the following composition: Na_2CO_3 , 0.25M; NaHCO_3 , 0.1M; NaCl , 0.1M. After cooling, the resulting complexes were coupled with *p*-dimethylaminobenzaldehyde in 0.5N-HCl containing 56% (v/v) of ethanol, and further ethanol was added to give 65% (v/v) ethanol, the final concentration of acid being 0.4N-HCl. The solutions were allowed to stand at 22.5° for 24 hr. so that the complexes attained their stable absorption spectra. Fig. 2 gives the absorption-spectrum curve of the complex from the phenol-soluble mucopolysaccharide. Similar curves were obtained with the phenol-insoluble mucopolysaccharide and the mucoprotein. Also given in Fig. 2 are the absorption-spectrum curves of the corresponding complexes obtained after treating glucosamine and chondrosamine with acetylacetone and coupling with *p*-dimethylaminobenzaldehyde. It may be seen that the absorption maximum of the complex from the phenol-soluble mucopolysaccharide lies between that of the glucosamine compound and that of the chondrosamine compound. The ab-

Fig. 2. Absorption-spectrum curve of the complex obtained by treating the hexosamines from the hydrolysate of the phenol-soluble mucopolysaccharide with acetylacetone and coupling with *p*-dimethylaminobenzaldehyde, compared with the absorption-spectra curves of the corresponding complexes obtained from glucosamine and chondrosamine. The λ_{max} for the complex from the phenol-soluble mucopolysaccharide hydrolysate (●) was $516\text{ m}\mu$, compared with the λ_{max} of the glucosamine complex (O), which was $512\text{ m}\mu$, and that of the chondrosamine complex (Δ), which was $535\text{ m}\mu$.

sorption curve of the complex produced by treating an equal mixture (w/w) of glucosamine and chondrosamine with acetylacetone and coupling with *p*-dimethylaminobenzaldehyde was found to have an absorption maximum at $523\text{ m}\mu$. Thus it was assumed that the difference between the absorption spectra of the complexes from hydrolysates of the mucopolysaccharides and the mucoprotein and that of the complex from glucosamine was due to chondrosamine, the probable presence of which was also indicated, with the mucopolysaccharides, by chromatography.

The sugars liberated by hydrolysis of the mucopolysaccharides and the mucoprotein with 0.5N-HCl were identified by filter-paper partition chromatography. Ascending chromatograms were run at 22.5° in four solvent systems and the hydrolysates were irrigated on the same papers as the controls: glucosamine, chondrosamine, galactose, mannose and fucose and with mixtures of these reference sugars. With the phenol-insoluble and the phenol-soluble mucopolysaccharides, the collidine-water system resolved the hydrolysates into four components: (1) R_f 0.19, corresponding to chondrosamine; (2) R_f 0.23, to glucosamine; (3) R_f 0.33, to galactose; (4) R_f 0.48, to fucose. Similar results were obtained with the pyridine-butanol-benzene-water system and the phenol-water system, except that neither of these solvent mixtures separated the two amino sugars. The butanol-acetic acid-water system was able only to separate fucose from the other sugars. The mucoprotein hydrolysate was resolved by the pyridine-butanol-benzene-water system into four components corresponding to the amino sugars (R_f 0.23), galactose (R_f 0.28), mannose (R_f 0.35) and fucose (R_f 0.46). The results obtained with the other three solvent systems confirmed the presence of these sugars in the hydrolysate, but no solvent system resolved the amino sugars, despite spectrophotometric evidence that was obtained of the presence of both glucosamine and chondrosamine. It was concluded therefore that the hydrolysates of the mucopolysaccharides contained glucosamine, chondrosamine, galactose and fucose, and that the hydrolysate of the mucoprotein contained glucosamine and possibly chondrosamine, galactose, mannose and fucose.

The amino acids liberated from the phenol-insoluble and phenol-soluble mucopolysaccharides by hydrolysis with 6N-HCl were identified by two-dimensional paper partition chromatography. Evidence was obtained of the presence of the following amino acids in the mucopolysaccharides: arginine, histidine, lysine, serine, threonine, leucine, isoleucine, valine, glutamic acid, aspartic acid, glycine, alanine and proline. Spots corresponding to cystine, tyrosine and phenylalanine were also

obtained, but these were not as clearly defined. Two other ninhydrin-reacting areas were observed in the chromatograms of both mucopolysaccharide hydrolysates, but these were never seen in chromatograms of mixtures of the series of amino acids listed above. The larger area was reproduced when amino acid mixtures containing hexosamines were chromatographed under the same conditions. The smaller area, R_f 0.05 in the butanol-acetic acid-water system and R_f 0.08 in the pyridine-butanol-benzene-water system, could not be identified.

Comparison of the properties of the mucoproteins of sputum and of urine

As the mucoprotein from sputum had a lower polysaccharide content than that of the sputum mucopolysaccharides and, unlike them, contained mannose, it appeared to resemble the mucoprotein isolated by Tamm & Horsfall (1952) from urine, rather than either of the mucopolysaccharides. This urinary mucoprotein was therefore prepared so that its properties could be compared directly with those of the mucoprotein from sputum.

Both complexes were obtained as voluminous white powders which gave neutral aqueous solutions. Both were precipitated when the pH of these aqueous solutions was lowered below 3.3. Electrophoretic examination showed that each complex was essentially homogeneous. On hydrolysis, both mucoproteins were found to contain amino sugars, and galactose, mannose and fucose. Both Gottschalk (1952) and Odin (1952) have found these sugars to be components of the carbohydrate residue of the urinary mucoprotein. Similar patterns were obtained when hydrolysates of the mucoproteins were irrigated with the pyridine-butanol-benzene-water mixture on the same ascending-paper chromatogram, though the fucose spot of the sputum-mucoprotein hydrolysate was more intense than the corresponding fucose spot of the urinary-mucoprotein hydrolysate.

The two complexes, however, differed in the following ways. The sputum mucoprotein was soluble in 0.58 M-NaCl, whereas the urinary mucoprotein was precipitated completely from its aqueous solutions by 0.58 M-NaCl. The viscosities of comparable solutions (Table 2) indicated that the urinary mucoprotein was probably a larger molecule than the sputum mucoprotein. The measurements were carried out in salt-free aqueous solutions of the two complexes because the presence of even low concentrations of inorganic salts may have caused the urinary mucoprotein to assume a heterogeneous disperse phase and thus to give rise to false viscosity readings. The relative viscosity of the sputum mucoprotein in water was much higher than the relative viscosity of the same complex in aq. 0.85 % NaCl soln. This difference may be

due to the electroviscous effect described by Bull (1940).

The ultraviolet-absorption spectra of the two complexes also differed (Fig. 3), the sputum mucoprotein showing a peak at 259 m μ and the urinary mucoprotein a peak at 278 m μ . In Table 2 it may

Table 2. Comparison of the composition and properties of the mucoproteins of sputum and of urine

Analyses of the mucoproteins were carried out on samples which were dried at 56° for 1 hr. The analytical results are expressed as a percentage of this dry weight. For methods of analysis, see text.

	Sputum mucoprotein	Urinary mucoprotein
Total N (%)	8.7	10.3
Amino sugars (%)	14.3	8.4
Reducing sugars (%)	26.3	16.7
Total P (%)	0.75	0.05
Inorganic P (%)	0.01	Nil
Organic P (%)	0.74	0.05
Relative viscosity (ν)*	5.31	5.81

* Viscosity measurements were made on 0.2 % solutions of the mucoproteins in water.

Fig. 3. Ultraviolet-absorption-spectra curves of 0.025 % solutions of the mucoproteins of sputum (O) and urine (●) in 0.025M- Na_2HPO_4 - NaH_2PO_4 buffer, pH 6.8. The λ_{max} for the sputum mucoprotein was 259 m μ and the λ_{max} for the urinary mucoprotein was 275 m μ .

be seen that this difference is reflected in the organic phosphorus contents of the two complexes and was possibly due to the presence of nucleic acids in the sputum mucoprotein. The analytical figures in Table 2 show that the sputum mucoprotein had a smaller total nitrogen content and a greater hexosamine and reducing-sugar content than the urinary mucoprotein had. The sputum mucoprotein was therefore a complex which contained approximately 50 % of polysaccharide, in contrast with the urinary mucoprotein which contained approximately 40 % of polysaccharide. The sputum mucoprotein was also shown to contain alkali-labile *N*-acetylhexosamine linkages (Morgan & Elson, 1934), whereas it was not possible to demonstrate the existence of such linkages in the urinary mucoprotein.

DISCUSSION

The method of isolating the mucopolysaccharides and the mucoprotein is suitable only for revealing the nature of the carbohydrate constituents of sputum. The total yield of material after peptic digestion, estimated on a weight for weight basis with acetone-dried sputum, was less than 40 %, indicating that these carbohydrate constituents probably make up less than half of the total solids present in pooled sputum. This approximate estimate is also in accord with that obtained from the analysis of acetone-dried sputum, the remainder of the high-molecular-weight components being either proteins or nucleoproteins. The enzymic digestion made it impossible also to obtain any information about the mechanism of coagulum formation. The method may, however, be considered adequate for isolating carbohydrate constituents, as it has been used to isolate blood-group mucopolysaccharides from saliva and from human stomachs (Kabat *et al.* 1947), the specific activities of these mucopolysaccharides being retained to a high titre. Similarly, the phenol-fractionation technique of Morgan & King (1943), which was used to purify the mucopolysaccharides, has been employed by Aminoff, Morgan & Watkins (1950) and Annison & Morgan (1952*a, b*) to purify blood-group mucopolysaccharides from ovarian-cyst mucin; their work has shown that the method has no deleterious effect on the specific activity of the complexes. Thus although the final products isolated by such procedures from sputum probably cannot be equated with the carbohydrates in their native state in the lower respiratory tract, they were a valuable guide for assessing the type of carbohydrate complex present.

Analysis showed that the two mucopolysaccharide preparations from sputum are very similar in composition to the various blood-group muco-

polysaccharides which have been isolated from human stomachs by Kabat *et al.* (1947), from meconium by Buchanan & Rapoport (1951) and from ovarian-cyst fluids by Aminoff *et al.* (1950) and Annison & Morgan (1952*a, b*). The component sugars of the carbohydrate moieties of the sputum mucopolysaccharides are identical with those of the blood-group mucopolysaccharides (Aminoff *et al.* 1950; Bendich, Kabat & Bezer, 1947). The sputum mucopolysaccharides possess alkali-labile *N*-acetylhexosamine linkages that are similar to those of the blood-group mucopolysaccharides (Aminoff *et al.* 1952). Furthermore, the amino acids of the polypeptide moiety of the sputum mucopolysaccharides are closely similar to those of the polypeptide moiety of the blood-group mucopolysaccharides (Aminoff *et al.* 1950; Annison & Morgan, 1952*a, b*). The finding of several molecular species of mucopolysaccharide in the pooled sputum obtained from a large number of patients also suggests that these complexes belong to the same class of substance as blood-group mucopolysaccharides, since considerable differences in molecular dimensions are found between type-specific mucopolysaccharides from different blood groups (Kekwick, 1952*a, b*).

In contrast with the several molecular species of mucopolysaccharide that were isolated from pooled sputum, only one essentially homogeneous mucoprotein was obtained. This complex is distinct in composition from the mucopolysaccharides and possesses different physical and chemical properties. It bears more resemblance to the mucoprotein that can be isolated from urine by the method of Tamm & Horsfall (1952) than to either of the sputum mucopolysaccharides. The polysaccharide moiety of the sputum mucoprotein is composed of the same sugars as that of the urinary mucoprotein, but the urinary mucoprotein does not possess the alkali-labile *N*-acetylhexosamine linkages that are present in the sputum mucoprotein. Analysis showed that the urinary mucoprotein has a larger polypeptide content than the sputum mucoprotein, and viscosity measurements indicated that the urinary mucoprotein is probably the larger molecule of the two. The sputum mucoprotein possibly contains nucleic acids which apparently form part of its molecular structure.

Some of these differences between the mucoprotein of sputum and that of urine may have been due to the manner of preparing the sputum mucoprotein, which involved enzymic digestion of acetone-dried sputum. This material contained nucleoprotein debris from the leucocytes and epithelial cells which are present, particularly in mucopurulent sputum. Not only may the enzymic digestion have had the possible effect of degrading the mucoprotein complex, but it could also have

released nucleic acids from denatured nucleoproteins of cellular origin which in turn, by election, may have formed a complex with the mucoprotein. The sputum mucoprotein in the native state therefore may resemble more closely the urinary mucoprotein so far as it may be of a molecular size more comparable with that of the complex from urine; in addition, it may not contain nucleic acids as part of its molecular architecture.

The question may be raised whether the mucopolysaccharides and the mucoprotein of sputum are present solely as an outcome of morbid change. Similar polysaccharides are produced at other sites by apparently normal epithelial surfaces which possess either goblet cells or the tubuloalveolar type of muco-serous glands, or both. These carbohydrate complexes which occur in sputum may therefore, either wholly or partly, be regarded as products of the secretory or hypersecretory activities of the bronchial epithelium, possibly modified by pathological changes.

SUMMARY

1. A method has been described for isolating two mucopolysaccharide fractions and an electrophoretically homogeneous mucoprotein from pooled acetone-dried sputum.

2. The two mucopolysaccharides, one insoluble and the other soluble in 90% (w/v) phenol-water mixture, contained 5.2 and 5.7% of nitrogen respectively; they were similar in composition to the blood-group mucopolysaccharides.

3. The mucoprotein, which contained 8.7% of nitrogen, was found to be distinct from either of the mucopolysaccharides isolated from sputum, but to have some properties in common with the mucoprotein that can be isolated from urine.

4. The polysaccharide moieties of the mucopolysaccharides were composed of *N*-acetylglucosamine, *N*-acetylchondrosamine, galactose and fucose; the mucoprotein contained mannose in addition to these sugars.

5. The phenol-insoluble mucopolysaccharide was electrophoretically non-homogeneous, showing two components which moved at similar velocities. In contrast, the sputum mucoprotein was essentially homogeneous.

6. These carbohydrate complexes constitute less than 50% of the total solids of pooled sputum. They represent, either wholly or partly, the secretory activities of the bronchial epithelium possibly modified by disease; they may have suffered some degree of degradation during their isolation.

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