in a large decrease in the Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} concentrations but in only a small decrease in the Cu^{2+} and Zn^{2+} concentrations, suggesting that these metal ions were firmly bound to one or more components of the mucus. Small but significant amounts of Ca^{2+} , Mg^{2+} and K^{+} also remained.

Gel filtration of the dialysed mucus on Sephadex G-75 and elution with deionized water gave an excluded fraction that retained Zn^{2+} and Cu^{2+} and that consisted mainly of glycoproteins low in sialic acid, aromatic and sulphur-containing amino acids, phosphate and sulphate.

In accord with the observation (Jones, 1938) that heavy-metal toxins in a fresh-water environment cause precipitation of gill mucus, low concentrations of Cu^{2+} , Zn^{2+} and Hg^{2+} dissolved in sea-water, when added to fresh plaice mucus, also cause precipitation in the order $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Hg}^{2+}$. Thus the interaction of these metal ions can produce a marked change in the properties of the mucus, and the cumulative effects of exposure of fish to a polluted environment could result in an impaired respiratory exchange as well as in other sublethal effects.

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Glycopeptides of the Sputum Gel Phase

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After proteolysis of the water-insoluble mucins of sputum, water-soluble glycopeptides may be isolated and fractionated according to their solubility at pH 2.8 (Brogan, 1959) and their sulphate content (Havez *et al.*, 1967). In the present investigation the sputum gel phase was subjected to proteolysis with trypsin after a preliminary digestion with deoxyribonuclease. After removal of the insoluble material from the tryptic-digest solution, the pH was lowered to 2.8 and the material that was precipitated out of solution was collected. The precipitate was redissolved in 50 mM-tris-acetate buffer, pH 7.3, and separated in a column of Sephadex G-200, and the material that was eluted in the void volume was collected and concentrated. The material was separated into two fractions by means of a column of Bio-Gel 15m agarose, and these were designated the acid-insoluble glycopeptides 1 and 2 according to their order of elution. The glycopeptides that remained in solution at pH 2.8 were fractionated according to their solubility in 0.3% Rivanol at various pH values. Three glycopeptide fractions were obtained: one was insoluble in 0.3% Rivanol at pH 1.5, one was pre-

cipitated at pH 5.0 and one remained in solution at pH 7.0. These glycopeptide fractions were designated the acid-soluble glycopeptides 1, 2 and 3 respectively. All three fractions were further purified by precipitation with 3 vol. of ethanol and finally by chromatography in Sephadex G-200. The five glycopeptide fractions were analysed for their amino acid, galactose, fucose, sialic acid, sulphate and phosphate contents. The acid-insoluble glycopeptides were characterized by their high content of sulphate (5–6%) and relatively low content of carbohydrate as compared with the acid-soluble glycopeptides. Analysis of the acid-soluble glycopeptides suggested that they might be classified as sulphosialoglycopeptide, sialoglycopeptide and fucoglycopeptides.

These findings confirmed the work of the histochemists, who have described three types of mucin-producing cells in the lung, those containing sulphosialomucins, sialomucins and fucomucins (Reid, 1965).

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Glycoasparagines in the Urine of Patients with Aspartylglycosaminuria

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Aspartylglycosaminuria is an inborn error of human metabolism associated with mental retardation, and is probably due to a lack of the enzyme responsible for the hydrolysis of aspartylglycosamine [2-acetamido-1-(β' -L-aspartamido)-1,2-dideoxy- β -D-glucose] (Pollitt *et al.*, 1968; Pollitt & Jenner, 1969). In addition to the aspartylglycosamine itself, the urine from our patients contains a large number of other compounds, giving a similar yellow-brown colour with ninhydrin and having higher molecular weights. As the condition possibly has storage aspects (Palo *et al.*, 1970; Pollitt & Jenner, 1971), it is of interest to determine the nature of these compounds.

The higher-molecular-weight compounds were isolated from the urine of one of our patients by using a combination of chromatography on Sephadex G-15 and either ion-exchange chromatography (Zeo-Karb 225, pH 2.0) or preparative paper electrophoresis. Internal standards of alanine and mannitol were added to the purified compounds, and portions were hydrolysed and examined on the amino acid analyser,