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

By H. C. RYLEY (*Department of Medical Microbiology, Welsh National School of Medicine, Cardiff CF4 4XN, U.K.*)

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

By R. J. POLLITT and KATHLEEN M. PRETTY (*Medical Research Council Unit for Metabolic Studies in Psychiatry, University Department of Psychiatry, Middlewood Hospital, P.O. Box 134, Sheffield S6 1TP, U.K.*)

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,

or methanolysed, reacylated and examined by g.l.c. as their trimethylsilyl derivatives (Clamp *et al.*, 1967).

The compounds fell into two classes, namely those that were neutral at pH 5.3 but separated on electrophoresis at pH 2.0, and acidic compounds, with very low electrophoretic mobilities at pH 2.0, but separable at pH 5.3. Three major neutral components were seen, namely components N₁, N₂ and N₃ with respectively one, two and three additional neutral carbohydrate residues attached to the aspartylglycosamine residue. Traces of higher-molecular-weight neutral compounds were also present. Component N₁ had an additional galactose residue, and component N₂ had galactose and fucose. Component N₃ could be resolved into two components on prolonged electrophoresis at pH 2.0; one had two mannose residues and one galactose residue, and the other had two mannose residues and one additional *N*-acetylglucosamine residue.

At least nine acidic compounds were present, with some members containing more than one acidic group/molecule. Most of these compounds contained sialic acid and were converted into neutral compounds on treatment with 0.1 M-HCl at 80°C; however, at least one of the more acidic compounds was stable to this treatment, and it possibly contained a sulphated residue as it moved slowly to the anode even at pH 2.0.

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The Iodination of Mammalian Cells Catalysed by Lactoperoxidase as a Probe for Externally Located Proteins of the Plasma Membrane

By ENID OWEN, V. A. KNIGHT and H. W. THOMAS
(Department of Physiology, University College, Cathays Park, Cardiff CF1 1XL, U.K.)

The use of lactoperoxidase to catalyse the iodination of tyrosine residues exposed on the outside of human erythrocyte membranes has been described by Phillips & Morrison (1971). We have been able to confirm their finding that only two peptide-chain components (as revealed by polyacrylamide gel

electrophoresis in sodium dodecyl sulphate) are labelled. One of these components is the glycoprotein of the membrane. In addition, we have obtained evidence that the sites on both components can saturate, and exhibit similar kinetic behaviour in this iodination reaction. Since the macromolecule lactoperoxidase must participate in this specific iodination, this reaction should provide an excellent means of identifying protein components of cells that could possibly participate in specific intercellular interactions. We are therefore applying this label to the case of the cultured BHK 21 cell, and preliminary results will be reported.

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Isolation of Plasma-Membrane-Associated Tumour-Specific Antigen from Rat Hepatoma Cells

By R. W. BALDWIN, J. R. HARRIS and M. R. PRICE
(Cancer Research Campaign Laboratories, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.)

Carcinogen-induced rat hepatomas and fibrosarcomas possess tumour-specific antigens, comparable with weak histocompatibility antigens, against which the host may evoke an immune response (Baldwin *et al.*, 1971*a,b*). These antigens are specific products of individual tumours and are viewed to be new discrete components of the transformed cell surface. Limited papain digestion of cell membranes has been used extensively to release H-2 and HL-A transplantation antigens as water-soluble glycoproteins (Reisfeld & Kahan, 1970). Similar techniques have now been applied to an amino-azodye-induced rat hepatoma (hepatoma D23) to characterize tumour-specific antigens.

Antigenic activity present in individual or pooled preparations of solubilized hepatoma D23 cell membranes was determined by their capacity to neutralize the reaction of antibody in hepatoma D23 immune serum with tumour-specific antigens expressed on viable hepatoma D23 cells. This inhibition of antibody reactivity was measured by using the indirect membrane immunofluorescence test (Baldwin & Barker, 1967).

An 'extranuclear' membrane preparation of hepatoma D23 tissue was subjected to limited papain digestion (1 mg of enzyme/30 mg of membrane protein) for 60 min at 37°C. The soluble extract was fractionated by DEAE-cellulose column chromatography by the technique of Baldwin & Glaves (1972). Velocity sedimentation of eluted fractions exhibiting