6 M-guanidinium chloride supplemented with proteinase inhibitors. The macromolecules were purified from proteins and lipids by isopycnic density-gradient centrifugation in CsCl/4M-guanidinium chloride and from DNA in CsCl/0.5 M-guanidinium chloride. In the latter gradient, mucins from the corpus and pylorus regions were heterogeneous with 'high' and 'low' density populations. Mucins from the cardiac region banded at an even higher buoyant density than both these components. Three populations of mucins were thus identified. The high density component from the corpus region contains more sulphate than the low density one as indicated by histochemical staining of mucins with the high-iron diamine (HID) reagent after slot blotting the macromolecules onto nitrocellulose membranes. In addition, the former component contains a higher ratio of sialic acid to hexose than the latter one. All surface mucous cells in the corpus region stain with periodic acid-Schiff (PAS) whereas only some show reactivity with HID. These findings indicate that the two mucin populations have different cellular origins.

Whole mucins from all regions eluted with the void volume of Sepharose CL-2B whereas reductive fragments (subunits) were included. Trypsin digestion caused a significant fragmentation of subunits and gastric mucins are thus composed of subunits that contain alternating highly glycosylated and sparsely substituted regions of the protein core. When subjected to ion-exchange h.p.l.c. on Mono Q, high-M_r glycopeptides from the 'high-density' mucins (the corpus and pylorus regions) elute later than those from the 'low-density' ones and both elute earlier than such glycopeptides from the cardiac region. A higher buoyant density of the whole mucins thus correlates to a higher charge density in the cognate glycopeptides. The negative charges in mucins are due to substitution with sialic acid and/or sulphate residues and the differences in charge density are thus due to differences in the oligosaccharide structures. In conclusion, the three mucins identified in pig stomach share a common macromolecular architecture but vary in their glycosylation.

S20.11
Comparative Study of the *O*-Linked Carbohydrate Chains Released from the Jelly Coat of Amphibian Eggs

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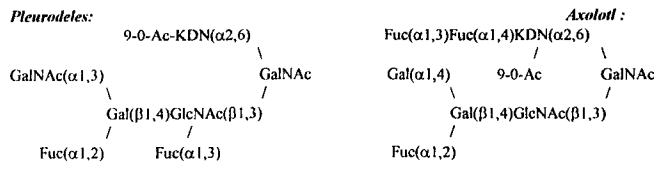
The *O*-glycanic chains of the mucins of *Pleurodeles waltl, Axolotl mexicanum, Xenopus loevis* and *Bufo Bufo* were released by reductive *b*-elimination and investigated by ^1H and ^{13}C-NMR spectroscopy. In *Axolotl, Pleurodeles* and *Xenopus*, KDN and 9-0-Ac KDN were found as the only Sia-derivatives, while NeuAx was exclusively observed in *Bufo*. The carbohydrate chains were found to be specific of each species. For example, in *Pleurodeles* and *Axolotl*, these main carbohydrate chain were observed:



In addition to the taxonomic interest of these observations, it is interesting to notify the role of these mucins in the fertilisation process.

S20.12
Analysis of Protein Portion of Porcine Gastric Mucus Glycoprotein After Release of *O*-Linked Oligosaccharide by Gas-Phase Hydrazinolysis

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Gas-phase hydrazinolysis was used to analyze the glycoform of *O*-glycan of fetuin and human myeloma immunoglobulin A1. It indicated that an *O*-linked oligosaccharide having a reducing terminal was released and their qualitative analysis was possible (1). However, details of this reaction are still not clear.

In this report, further estimation of this method was carried out using porcine gastric mucus glycoprotein (PGM). Amino acid analyses of the original PGM and recovered PGM after the reaction indicated that the recovery of serine (73%) and threonine (71%) was lower than those of other rich amino acids (91% – 107%) in PGM. The selective decrease in both amino acids indicated that they were converted to the corresponding unsaturated amino acid by a β-elimination reaction. The presence of dehydroalanine in treated PGM was confirmed by its conversion to cysteic acid by the reaction with sodium sulfite. This differed from a recent report on the threonine residue specificity for the protein receptor of purified *N*-acetylgalactosaminyl transferase from the submaxillary gland, the observed coincidence of percent recovery for both amino acids may indicate an equal distribution of the carbohydrate chain on serine and threonine in PGM. The release of oligosaccharide increased to 77% when compared with conventional sodium borohydride treatment. However, galactose and *N*-acetylgalactosamine added to bovine serum albumin were found to be partly decomposed during the reaction. The previous failure to detect free *N*-acetylgalactosamine from the IgA1 molecule was due to its small content and by such a side reaction (1).

Despite these shortcomings to be overcome, this method would be important as the only chemical method to prepare an *O*-linked oligosaccharide having a reducing terminal from glycoproteins.

(1) H. Iwase *et al. Anal. Biochem.*, **206**, 202 – 205 (1992)

S20.13
A Monoclonal Antibody Against Mucin Derived from Rat Gastric Surface Epithelial Cells and Partial Characterization of the Epitope Structure by Enzyme Immunoassay

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In the mammalian gastric mucosa, mucus-secreting cells have