The Carbohydrate Residues of Gastric Mucins in Fetal and Adult Rats

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The carbohydrate residues of gastric mucins were examined by various lectins. The sections (2 μm in thickness) were reacted with biotinyl-lectins, and were reacted with FITC-labeled avidin. The reaction sites of each lectin were clearly observed as the specific fluorescence. By this method, gastric mucins containing in surface epithelium were different from them in mucous neck cells in the composition of carbohydrate residues; the mucins in surface epithelium contained β-D-galactosamine, α-L-fucosyl, and β-D-glucosamine. But the sugar residues of the mucins in mucous neck cells were mainly β-D-galactose. The gastric mucin of adult rat stomach did not contain α-D-galactose residue. But, α-D-galactose residue was clearly recognized in the gastric mucous epithelium both of fetal (17-20 days of gestation) and of newborn rat (0-9 days after birth).

Modified PAS reactions for demonstrating different sialic acids

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Many different species of sialic acid have now been isolated and identified biochemically. Reliable histochemical techniques for selective demonstration of different sialic acids, however, have been restricted to sialidase digestion followed by basic dye stainings. By employing modified PAS reactions, the present study was undertaken to elucidate the distribution of N-acetylneuraminic acid(NANA), 8-O-acetyl NANA, 7-O-acetyl NANA and 9-O-acetyl NANA in the human gastrointestinal tract.

(1)difinite selective staining of NANA was obtained by mild periodate oxidation(1mM, 0°C, 10min)-Schiff reaction. (2)8-O-acetyl NANA was demonstrated by modified Culling's sequence(PA-RED-ALH-PAS). (3)7-O-acetyl NANA should show equal ordinary PAS and PA-RED-ALH-PAS reactivity. (4) C_{7-8} vicinal hydroxyl in 9-O-acetyl NANA is only oxidized by prolonged periodate oxidation for 48hrs and, subsequently, would lose routine PA-RED-ALH-PAS reactivity.

The results revealed that:(1)reactivity to NANA and 8-O-acetyl NANA was inversely propertional in any gastrointestinal mucous cells. (2)9-O-acetyl NANA was not present in the intestines.

Histochemical application of prolonged mild alkaline hydrolysis for differentiation of mucin-type glycoproteins

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Glycoproteins have been classified into three major types based on their glycopeptide bonds. Among them, only O-glycosidic linkage between N-acetylgalactosamine and serine or threonine are susceptible to alkaline hydrolysis (AH). Biochemical AH is performed under relatively severe conditions and, thus, was hardly applicable to histochemical staining. In the present study, the authors have developed a new sequence in which tissue sections were collodionized before and, in addition, after AH with 0.5 M NaOH in 70% ethanol and the effect of AH on mucosubstances were examined by PAS reaction.

Exposure to AH for 48-144 hours led to a complete loss of PAS reactivity of epithelial mucins in rat sublingual glands, stomach, small intestine and large intestine, in order of decreasing susceptibility, whereas that of surface coat-type mucins, thyroid colloid, collagen and cartilage were well preserved. These findings agreed with biochemical analyses previously reported. The present study also revealed that materials prepared by freeze-substitution technique provided the most satisfactory results.

A Dual Staining Method for Complex Carbohydrates with Alkaline Phosphatase-labeled Concanavalin A (Con A-ALP) and Periodic Acid-Schiff (PAS)

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A dual staining method has been developed for the histochemical demonstration of α-D-mannosyl and α-D-glucosyl residues and 1,2-glycol groups of neutral complex carbohydrates in light microscopy. It combines an alkaline phosphatase-labeled concanavalin A-5-bromo-3-indolyl phosphate, p-toluidine salt (Con A-ALP-BIPT) method with a periodic acid-Schiff (PAS) sequence. A series of organs from various animal species were employed as materials; umbilical cord (pig, cattle), aorta (pig, cattle, monkey), comb (cock), stomach (monkey), jejunum (monkey), colon (monkey), submaxillary gland (pig) and sublingual gland (cattle). These tissues were routinely fixed, dehydrated, paraffin-embedded, sectioned, deparaffinized and subjected to the Con A-ALP-BIPT-PAS staining. With this method, it is possible to stain α-D-mannosyl and α-D-glucosyl residues blue and 1,2-glycol groups of neutral complex carbohydrates magenta. The validity of the method has been confirmed with appropriate histochemical controls on test tissues.