ACTA CHEMICA SCANDINAVICA 19 (1965) 797–799

The Fractionation and Characterisation of the Acid Polysaccharides in Human Gastric Juice

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Gastric juice was collected from healthy male individuals after histamine administration. The acid polysaccharides in the gastric juice were isolated, digested with papain and fractionated by precipitation with cetylpyridinium chloride and stepwise salt elution on cellulose columns. Four different fractions were obtained that were homogeneous by all criteria. None of them contained hexuronic acids and hence there was no contamination by connective tissue polysaccharides. All fractions contained sulphate in amounts increasing from the first to the last fraction.

Gastric juice has been found to contain sulphate.[1] In animal experiments it has been found that radioactive sulphate partly incorporated in non-dialysable substances migrates from the gastric wall to the gastric juice.[2,3] Aminosugars, hexoses and fucose have also been found in gastric juice.[4,5] The sulphate-containing macromolecules in gastric juice have not been identified in any of these investigations. The materials from which these gastric macromolecules have been analysed have possibly been contaminated by tissue. Perhaps it is due to this that the nature of "mucoitin sulphuric acid" is still obscure.[6]

We have noted that secreted gastric polysaccharides and those released from connective tissue very readily mix and are difficult to separate completely by alcohol precipitation.[7]

With newer methods we have managed to isolate all the acid polysaccharides in the gastric wall of the dog in a pure enough form to permit their characterisation.[8] In connection with this characterisation we wished to study which polyanions were definitely epithelial, *i.e.* secreted products. We detected four sulphate containing polysaccharide fractions with similar properties.[8] These fractions differed completely from the classical idea of a sulphopolysaccharide. In the work described below similar analyses were performed on human gastric juice.

MATERIAL AND METHODS

Eight voluntary and healthy young male medical students were given 1 ml of Histalog (Lilly Co.) subcutaneously after their stomachs had been emptied through an orally introduced tube. The gastric juices were collected during a period of one hour. The juices were centrifuged and pooled, whereupon 1.5 litres of gastric juice was obtained. The juice was neutralised with sodium bicarbonate, freeze-dried immediately, and stored as a dry powder for later analysis.

The polyanions were liberated from their protein complexes by digestion with papain as described elsewhere [8] using 12 mg of twice crystallized papain to 400 mg of gastric powder. The polyanions were precipitated with cetylpyridinium chloride (Recip, Stockholm) and then with alcohol and fractionated on cellulose columns as described in an earlier paper on the polyanions in gastric tissue.[8]

The analytical methods were the same as were used earlier.[8] The infra-red spectrum of the powder was recorded and its electrophoresis on cellulose acetate was studied. The powder was analysed for neutral sugars, aminosugars, uronic acids, and sialic acids by paper chromatography after hydrolysis with Dowex-50 and separation of the monosaccharides by ion exchange chromatography. The hexosamines, sulphate, sialic acids, galactose and fucose were analysed colorimetrically.

RESULTS AND DISCUSSION

The gastric juice always contains small amounts of tissue residues, the greater part of which can be removed by centrifugation but this contamination is not of any greater importance when the juice is collected over short periods.

The material precipitated with cetylpyridinium chloride after papain digestion was divided into four fractions by stepwise elution with salt solution from cellulose columns. The salt solutions were 0.3 N sodium chloride and 0.5 N, 0.6 N, and 0.8 N magnesium chloride solutions. Each subfraction weighed between 10 and 20 mg and gave one band on electrophoresis. On refractionation every fraction was eluted in the same way as after the first fractionation. Small amounts of other fractions were noted, but no nucleic acids were detected. The fraction eluted by 0.3 N sodium chloride did not contain the subfraction B found in the gastric wall.[8] All carbohydrates were eluted by 0.8 N magnesium chloride. The polysaccharide fractions in the gastric wall of the dog eluted by 1.0 N and 1.2 N magnesium chloride were not present in the human gastric juice.

The infra-red spectra of the fractions are identical in all respects except for the intensity of the sulphate peak (1250 cm^{-1}), which increased in the order of elution of the fractions. The spectra are very similar to the spectra of the fractions in the gastric wall of the dog that were eluted by 0.3 N sodium

Table 1. The ratios of glucosamine to galactosamine in polysaccharide fractions in gastric juice as calculated from aminosugar contents after ion exchange chromatographic separation.

Fraction	Glucosamine	Galactosamine
0.3	2.02	1.00
0.5	1.90	1.00
0.6	1.93	1.00
0.8	2.28	1.00

Table 2. Acid polysaccharide fractions in human gastric juice. Contents of aminosugars, galactose, fucose, sialic acids, and sulphate (a) inμg/μg aminosugars and (b) as molar ratios.

a) Fraction	Fucose	Galactose	Aminosugar	Sialic acid	-SO_4
0.3	50.5	72.5	100.0	16.0	6.25
0.5	48.0	71.0	100.0	8.8	12.0
0.6	47.0	76.5	100.0	9.6	22.0
0.8	49.2	66.7	100.0	11.4	23.8
b) Fraction	Fucose	Galactose	Aminosugar	Sialic acid	-SO_4
0.3	1.11	1.46	2.02	0.187	0.234
0.5	1.05	1.41	2.00	0.104	0.446
0.6	0.94	1.39	1.83	0.101	0.750
0.8	0.99	1.22	1.85	0.122	0.820

chloride (subfraction A) and 0.5 N, 0.6 N, and 0.8 N magnesium chloride solutions.[8]

All fractions were found to contain hexosamines, galactose, fucose, and sialic acids by paper chromatography. No uronic acids were detected in any of the fractions.

Every fraction contained both glucosamine and galactosamine. The ratios of glucosamine to galactosamine calculated from the quantitative data for hexosamines after their separation by ion exchange chromatography are shown in Table1. The ratio of glucosamine to galactosamine was very close to 2:1 in all fractions. This ratio was 1:1 in all the corresponding polysaccharide fractions in the gastric wall of the dog.[8] The difference can be ascribed to the different species.

The colorimetric data are shown in Table 2. Every fraction contained sulphate ion, the amount of which increased in successive fractions. The relative contents of hexosamine, fucose, galactose and sialic acids were the same in all the fractions.

The similarity of these fractions in both dog and man is obvious and it is possible that these fractions represent a secreted polyanion group.

Acknowledgement. This study has been supported by grant AM-6018 from the *National Institutes of Health, United States Public Health Service.*

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Received January 9, 1965.