Biochemical Studies on Carbohydrates.

CXIX. Hexosamine Compounds in Human Gastric Juice.

Third Communication:

Non-anti-hemoisoagglutinative Carbohydrates in A and B Gastric Juice.

By

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The carbohydrates N.A.A.C.'s from A and B gastric juice (cf. the first communication¹⁾) showed close resemblance mutually not only in composition but also in optical property. We incline thus to assume them identical with each other. Although these products exhibited faint anti-isohemoagglutinative potency specifically, it probably owed to imperfect removal of the group carbohydrate A.A.C. during the fractionation. The substances were difficultly soluble in plain water and dilute acid (0.01 n. HCl) but dissolved in dilute alkali.

EXPERIMENTAL.

Analysis and Optical Measurement.

The quantitative-analytical figures of the N.A.A.C.'s, which were obtained by our usual determination methods, are embodied in Table I. The molecular ratios of glucosamine, galactose and sulfuric acid in these polysaccharides approximate to 10:15:2, whereas the corresponding ratios in the group carbohydrates were found 10:10:1 (cf. the second communication²⁾).

The N.A.A.C.'s had similar optical rotations (The substances were dissolved in a dilute Na_2CO_3 solution at pH 8.6):

N.A.A.C. from A gastric juice (pH of the solution, 8.6):

$$[\alpha]_{\text{D}}^{26} = \frac{-0.44 \times 2.9833}{0.0358 \times 1.006 \times 1} = -36.45^\circ.$$

N.A.A.C. from B gastric juice (pH of the solution, 8.6):

$$[\alpha]_{\text{D}}^{19} = \frac{-0.23 \times 2.9911}{0.0184 \times 1.008 \times 1} = -37.09^\circ.$$

Identification of the Monosaccharide Components.

TABLE I.
Composition of N.A.A.C.'s from A and B Human Gastric Juice.
(g. j.=gastric juice)

Analysis	In per cent		In equivalent ratio	
	N.A.A.C. fr. A g.j.	N.A.A.C. fr. B g.j.	N.A.A.C. fr. A g.j.	N.A.A.C. fr. B g.j.
Nitrogen*	3.7	4.35	1.59	1.97
Glucosamine†	29.7	30.9	1.0	1.0
Acetyl‡	7.49	7.49	1.05	1.10
Galactose				
Indole r.§	38.9			
Orcinol r.	38.4	39.1		
Diphenylamine r. I§	38.5	39.7		
Mean	38.6	39.4	1.29	1.39
Hydrolysable ST	1.23	1.23	0.23	0.24
Ash**		2.1		

* Micro Kjeldahl. † Hamasato-Akakura method.

‡ M. Suzuki's modification of Friedrich, Rapoport and Sternberg method.

§ Dische method. // Tillmans and Philippi method simplified by Ozaki.

¶ BaSO₄ method under conditions preferred by M. Suzuki. ** Micro analysis.

N.A.A.C. from A gastric juice.

For the purpose the procedure in the 97th information of this series³¹ was adapted, namely, the hydrolysate by sulfuric acid was fractioned by means of mercuric oxide, and the hexosamine was identified as penta-benzoate with glucosamine and, as the colorimetric figures in Table I had predicted, the hexose as methylphenylhydrazone with galactose.

From 175 mgm. of the polysaccharide were 54 mgm. of crude hexosamine pentabenzoate obtained. After reprecipitations from methanol, the crystals with a constant melting point of 214.5–215°C. were examined regarding optical rotation and nitrogen content.

$$[\alpha]_D^{14}(\text{in pyridine}) = \frac{0.54 \times 1.4856}{0.0182 \times 0.984 \times 1} = +44.8^\circ.$$

N (micro Kjeldahl).

8.86 mgm. substance, 0.1427 mgm. N.

(COC₆H₅)₅C₆H₈O₅N. Found, 2.08%; calc., 2.00%.

Crude galactose methylphenylhydrazone (needles, 48 mgm.) obtained was recrystallized from absolute alcohol four times. By the final recrystallization, the melting point was not raised. Yield, 26 mgm. F.P., 190°C. Mixed melting point test showed no depression.

N (micro Dumas).

3.565 mgm. substance, 0.297 cc. N₂ (20.4°C., 756.99 mm. Hg).

C₁₃H₂₀O₅N₂. Found, 9.66%; calc., 9.85%.

N.A.A.C. from B gastric juice.

In this case hydrolysis was made by means of hydrochloric acid.

121 mgm. of the polysaccharide were heated with 10 cc. of 5.5 n. hydrochloric acid in a boiling water-bath for five hours. The appearing dark sediment was centrifuged off. The yellowish supernatant liquid was transferred to a porcelain dish, and concentrated on a water-bath to a syrup and 10 cc. of water added with following filtration. The filtrate was precipitated with 6 cc. of the saturated lead acetate. The centrifuged supernatant fluid was bubbled with H_2S gas, removed from lead sulfide, and after evaporation to about 8 cc., extracted with ether in a continuous extractor for three hours. Ether was aerated off. The liquid obtained was shaken with 7 cc. of 10% NaOH and 1 cc. of benzoylchloride under cooling in ice. The precipitates were washed with water exhaustively to free from alkali and crystallized from 95% alcohol (a small part insoluble in hot alcohol was discarded) and from methanol. The yield at this stage was 38 mgm. and F. P. $208^{\circ}C$. Recrystallized three times from methanol, it melted constant at $215-215.5^{\circ}C$. The amount of substance was reduced to 21 mgm. Crystalline form, thin needle.

N (micro Kjeldahl), 2.09% (5.65 mgm. substance, 0.118 mgm. N).

Calc. for glucosamine pentabenzate, 2.00%.

$$[\alpha]_D^{12}(\text{in pyridine}) = \frac{0.240 \times 2.9955}{0.0162 \times 0.9830 \times 1} = +45.16^{\circ}.$$

SUMMARY.

1. N.A.A.C.'s from A and B gastric juice were found identical with each other.
2. It is composed of glucosamine, galactose and sulfuric and acetic acid in molecular ratios of nearly 10:15:2:10.
3. It has the rotation $[\alpha]_D$ of $-36 \sim -37^{\circ}$.

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References.

- 1) Tiba, Teramura and Gotoh, Tohoku J. Exp. Med., 1949, 50, 287.
 - 2) Tiba, *ibid.*, 1949, 50, 293.
 - 3) Tiba, *ibid.*, 1949, 50, 281.
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