Hexosamine Compounds in Human Gastric Juice.

First Communication: Fractionation of Gastric Juice*.

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One decade and a half ago, Babkin and his collaborators¹⁾ pronounced that "dissolved" mucin involving Levene's mucoitinsulfuric acid is present in canine gastric juice²⁾ and furthermore that free mucoitinsulfuric acid unassociated by protein is not present in this secretion. On the other hand, it has been known among serologists since 1927 that the blood group substances are secreted in human gastric juice³⁾ and recently Witebsky and Klendshoj⁴⁾ isolated a "carbohydrate-like" substance possessing B property from gastric juice of patients belonging to this group. These group substances might be also conjectured to contain hexosamine, when analogized from what has been revealed with respect to those from other sources⁵⁾.

Taking the above informations into consideration, we have made trials to separate the individual hexosamine compounds in a pure state. The procedure which will be described in the experimental part appeared most simple and expedient. From each A and B gastric juice were obtained three kinds of substance. Two of them resembled mutually in biuret-negativeness, in strong Molisch-positiveness and in giving a red dye with dimethylaminobenzaldehyde, when heated in advance with alkali (Osaki and Turumi procedure⁶⁾), but diverged in inhibiting isoa-

* The 98th report of Biochemical Studies on Carbohydrates, Masamune.

glutination of erythrocytes extremely, one exhibiting only very faint activity probably due to contamination by the other. The third substance gave all those chemical reactions but possessed no anti-agglutinative property. Detailed studies, chemical and physical, of the preparations will be accounted for in following papers. It should be worthy to note here that we could not demonstrate the naphthoresorcinol reaction for hexuronic acid (Neuberg and Saneyoshi) neither in those final substances nor in the crude fractions nor in the starting material which had been precipitated from condensed gastric juice with alcohol.

EXPERIMENTAL.

Procedure of Fractionating Hexosamine Compounds in Gastric Juice.

Human gastric juice was collected from patients by the aid of a caffeine test solution (Katsch method). The diluted juice (pH 1.2–4.6) from each patient was first percolated (repeatedly two or three times) through a gauze and then made clear by filtration through a creased paper, after pH had been shifted, when necessary, to 4.2–4.6 with a saturated sodium acetate solution (turbidity increase was hardly noticed). The filtrate was evaporated to syrupy consistency under reduced pressure and 95% alcohol was added. The precipitates were washed with absolute alcohol and with ether and placed in a vacuum desiccator. The dried powder⁷⁾ amounted to 144 and 112 mgm. in averages respectively per 100 cc. of A and B juice. The group was determined on these materials. Later operations will be described taking a typical example started with those obtained from A group juice and united.

1.9 gm. of the powder were mixed well with 20 cc. of ethylene glycol and centrifuged over and over to get a transparent supernatant liquid. The centrifugate was again treated with 10 cc. of glycol similarly and the residue (α -Fraction) was washed with alcohol and ether and set aside—900 mgm. To the fluids combined was added as much absolute alcohol as to throw down all the precipitable substances. The latter were separated and after washing and drying as above (320 mgm), again agitated with three times the weight of water. The mixture was acidified to pH 4.2 with drop by drop of 30% acetic acid and prolonged centrifugation exerted. The supernatant clear liquid was condensed to half the volume at a low temperature and 10 volumes of glacial acetic acid (96%) added. The appearing precipitates were centrifuged, washed once with glacial acetic acid, many times with absolute alcohol to eliminate the whole acetic acid and finally with ether and dried (β -Fraction)—180 mgm. From the supernatant fluid here was thrown down a further fraction (γ -Fraction) by means of

98% alcohol (7 volumes were required for complete precipitation), washed with alcohol and ether as before and dried —100 mgm.

The yields of the β and γ fractions diverged widely from one run to another but the amounts per 100 cc. of gastric juice of the final substances obtained in the manner below were resembling independently from the run and also from the blood group.

Purification.

β -Fraction (180 mgm.). 10 cc. of water were used for dissolution. Although centrifugation was repeated, the supernatant liquid remained turbid. Hence this was precipitated with alcohol (95%, 60 cc.), and after washing with alcohol and ether and drying, again taken up in water (5 cc.). This time a clear supernatant liquid was obtained on centrifugation. The substance separated and dried as above was then freed from glycol-insoluble impurities by the aid of 10 cc. of this solvent. Precipitates were gained from the clear solution again by alcohol (95%, 70 cc.) and washed and dried similar to above. Finally they were taken up (a perfect solution was given) in 5 cc. of water and shaken with 75 cc. of glacial acetic acid (96%) for two days on an apparatus. The undissolved part was collected, washed with glacial acetic acid once, with many changes of 95% alcohol and with ether and dried. White powder amounting to 70 mgm. was obtained (N.A.A.C.).

γ -Fraction (100 mgm.). It was taken up in 5 cc. of water and the insoluble residue centrifuged off. To the opalescent supernatant liquid, which was not to be readily clarified by centrifugation, were added 2 cc. of 30% acetic acid. The turbidity-causing substance was thereby changed into flocculent precipitates, which were separated off by centrifugation. Alcohol (60 cc.) was added to the clear supernatant liquid and what was precipitated was washed with alcohol and ether and dried. Thereupon followed thorough mixing with 10 cc. of glycol and subsequent centrifugation. The supernatant liquid here was also precipitated with 95% alcohol (70 cc.) and the precipitates washed and dried likely. A single repetition of glycol treatment sufficed to remove all the substance insoluble in this fluid. Non-coloured powder produced was 63 mgm. (A.A.C.).

α -Fraction (900 mgm.). This was not exhaustively purified:—500 mgm. of this fraction were at first kneaded with a small volume of glycol and later agitated with the increased solvent (30 parts in total) and centrifuged. The centrifugate was washed with absolute alcohol, and dried after replacing alcohol with ether. 350 mgm. of brownish powder were yielded.

Chemical Properties and Serological Activity of the Products.

N.A.A.C.'s (Non-Antiagglutinative Carbohydrate).

They did not dissolve in water (only swelled), but did when neutralized. Biuret, ninhydrin, aniline acetate (for pentose), naphthoresorcinol (for hexuronic acid) and ammonium molybdate (for phosphorus) tests resulted all in negative. They showed intensive Molisch reaction (violet coloration). Dimethylaminobenzaldehyde reaction (Osaki and Turumi procedure) occurred plainly after heating of their alkaline solution but scarcely when this preliminary treatment was omitted. Hydrolysable sulfur (BaSO_4 method) was detected. Arginin (Sakaguchi) was present but histidine (Ehrlich diazo) absent.

The anti-isoagglutinative power was measured after Kobayasi⁸⁾. The employed A and B sera possessed titres 160 and 80 respectively. Both of the substances from A and B stomach juice showed group-specific activity only up to 1:1,000 dilution (10 times dilution of the original solution).

A.A.C.'s (Anti-Agglutinative Carbohydrate).

Qualitative chemical tests gave similar results as in the former polysaccharides. The substances from the both groups of stomach juices inhibited the agglutination specifically up to 1:1,000,000 dilution. The sera had the same titres as those in the preceding case.

The α -Fractions Purified with Glycol.

Biuret and Molisch reaction were shown. Direct Osaki and Turumi test, negative; the indirect (test for acetylhexosamine), positive. Hydrolysable sulfur was detected. Aniline acetate test for pentose was given although naphthoresorcinol test for hexuronic acid was not. The presence of phosphorus was proved. The substance was insoluble in water and even when shaken with a sodium carbonate solution, most of it remained undissolved and in this part was found a large quantity of phosphorus. The substance was also insoluble in dilute hydrochloric acid.

The serological assay indicated them non-anti-isoagglutinative.

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 - 7) This was coloured with methylene blue added to the caffeine test solution.
 - 8) Kobayasi, *J. Bioch.*, 1941, **33**, 369.
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