Biochemical Studies on Carbohydrates.

LXXXVIII. Does Mucoitin Exist in the Animal Body as Combined with Sulfuric Acid?

Third Report.

“Limacoitinsulfuric Acid” from Mucus-Mucin of Snail and Constitution of this Glycoprotein.*

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The claim of Levene¹⁾ that mucoitinsulfuric acid exists in the mucus-mucin of snail was assumed by H. Suzuki²⁾ as probable. To acquire a more exact knowledge of it, we submitted this glycoprotein again to the formaldehyde treatment. The product obtained was composed of acetylhexosamine (Osaki and Turumi test³⁾), uronic acid (Goldschmiedt and naphthoresorcinol tests) and sulfuric acid. Since Suzuki had been able to separate glucosamine as pentabenzoyl but not chondrosamine from the present mucin, we felt at first as if mucoitinsulfuric acid had been isolated. A further study, however, revealed that it gives colours with the indole and orcinol reagents in respective intensities coinciding mutually as expressed as galacturonic acid but not as expressed as glucuronic acid and that it rotates D-light to the right contrary to chondroitinsulfuric acid and mucoitin. In other words, it was found to be a new itinsulfuric acid, which we designated “limacoitinsulfuric acid.”

The residue of the mucus-mucin was recovered by heating its methylene derivative with 0.05 N. HCl according to Sasaki⁴⁾. The substance not only analyzed quantitatively similar to the foot-mucin prepared by Suzuki with respect to carbohydrate and nitrogen but also possessed a resembling optical rotatory power. It was also proved free from hydrolysable sulfur as

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is the foot-mucin. We incline to believe that the mucus-mucin of snail is made up of foot-mucin and limacoitinsulfuric acid.

EXPERIMENTAL.

Preparation Procedures.

Limacoitinsulfuric acid.

The mucus-mucin was prepared similarly as was by H. Suzuki, and 9 gm. of it were treated with formaldehyde in an alkaline solution by strictly following the processes which Osaki and Hasegawa⁵⁾ applied to umbilical mucin. After acidification and centrifugation, alcoholic precipitates amounting 700 mgm., when dry, were obtained from the supernatant liquid. The centrifugate after acidification was subjected to a repeated operation and set aside, after washing with 1% acetic acid, alcohol and ether and drying.

Limacoitinsulfuric acid was extracted three times from the alcohol precipitates by shaking the latter with 45 cc. of water and 15 cc. of an amylalcohol-chloroform (1:2 by volume) mixture overnight with following centrifugation. The supernatant fluids were combined altogether and shaken repeatedly with portions of the above organic mixture until no jelly formed. The final turbid solution, that could not be made transparent by passing through paper pulp⁶⁾, was acidified to pH 4.5 with a dilute acetic acid, agitated with 1 spoonful of purified Kahlbaum kaolin and brought onto a thin layer of the kaolin powder in a Büchner funnel. To the clear filtrate were then added 15 volumes of glacial acetic acid and 2 spoonfuls of crystalline sodium acetate (powdered), whereupon gelatinous precipitates appeared. The precipitates were centrifuged, washed three times with 95% alcohol and once with ether and dried in vacuum (P_2O_5). The crop of the white powder obtained was 250 mgm. Molisch reagents produced a green and an adjacent red zone. Neither repetition of mere passing through kaolin⁷⁾ nor treatment with phosphotungstic acid and kaolin⁸⁾ could perfectly remove the red-dye-giving contaminant. So finally the substance was electrodialyzed (Pauli apparatus, 200 volts), as dissolved in 30 cc. of water, for 5 days against many changes of water.

The solution (Molisch, green) and the dialysate in the anode compartment (Molisch, red) were condensed separately to about 15 cc. and 15 volumes of glacial acetic acid and sodium acetate were added. Deposits obtained were washed with acetic acid (glacial acetic acid diluted with 1/15 the volume of water), alcohol saturated with NaCl, plain alcohol and an alcohol-ether mixture in turn. The products both gave a green zone alone by Molisch reagents. They were united and reprecipitated from a little volume of water, washed and dried in a like way as above. Crop, 110 mgm.

The remaining part of the mucus-mucin.

400 mgm. from the acid-insoluble fraction (3.9 gm.) set aside in the above were agitated with 120 cc. of 0.05 n. hydrochloric acid at 100°C (under reflux and in a boiling water-bath). After 1 hour, the mixture was cooled and the insoluble part was centrifuged off. To the water-clear solution, whose pH was 1.8, was added 1 n. sodium carbonate solution to pH 6.2, whereby gelatinous precipitates appeared. The precipitates were collected by centrifugation, washed with water, alcohol and ether and dried in vacuum (P₂O₅). The product (white powder) weighed 139 mgm.

Physical and Chemical Properties.

Limacotinsulfuric acid.

It was hygroscopic and transparently soluble in water. Molisch, green. The naphthoresorcinol test for hexuronic acid was given. Hydrolysable sulfur was present in a large amount: Quantitative-analytical figures obtained are embodied in Table I.

TABLE I

	In per cent	In equivalents per equivalent weight
Nitrogen*	2.72	1.04
Glucosamine	33.59	1.00
Hexuronic Acid		
As glucuronic acid		
Indole r.	65.66	
Orcinol r.	33.76	
As galacturonic acid†		
Indole r.	37.93	
Orcinol r.	37.92	
Average	37.93	1.04
Hydrolysable sulfur ...	6.15	1.02
Acetyl	7.25	0.90
Ash‡	19.13	

TABLE II

	In per cent	In equivalents per equivalent weight
Nitrogen	14.09	
Glucosamine	4.36	1.00
Galactose		
Indole r.	4.12	
Orcinol r.	4.16	
Average	4.14	0.94
Acetyl	1.09	1.04
NH ₂ -N*	0.76	
Ash	0.76	

* Micro Dumas. † Ref. to Masamune, Suzuki and Kondoh. ‡ Micro analysis. * Micro Van Slyke method (shaken for 30 minutes). The corresponding value in foot-mucin, 0.74%.

Optical rotation in water:

$$[\alpha]_D^{19} = \frac{0.381 \times 1.2052}{0.00466 \times 1.0009 \times 1} = +98.5^\circ$$

The part of the mucus-mucin other than limacotinsulfuric acid.

It was insoluble in water, but dissolved when alkali was added. Biuret, positive. Molisch, violet. Hexuronic acid (naphthoresorcinol test) and hydrolysable sulfur were not detected. Table II above shows the quantitative data.

Optical rotation.

The substance was dissolved in water with the aid of 0.1 n. NaOH and pH of the solution was adjusted to 7.0 with 0.1 n. HCl.

$$[\alpha]_D^{16} = \frac{-0.401 \times 2.8248}{0.0198 \times 1.0009 \times 1} = -57^\circ \left(\text{Foot-mucin: } [\alpha]_D^{17} = -56^\circ \right)$$

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Bibliography and Notes.

- 1) Levene, J, Biol. Chem., 1925, **65**, 683.
- 2) Suzuki, H., J. Bioch., 1941, **33**, 377.
- 3) Osaki and Turumi, Tohoku J. Exp. Med., this volume p. 11.
- 4) Sasaki, Tohoku J. Exp. Med., 1945, **48**, 307.
- 5) Osaki and Hasegawa, Tohoku J. Exp. Med., 1943, **45**, 176.
- 6) With suction.
- 7) The substance was dissolved in 20 cc. of water, acidified to pH 4-5 with 30% acetic acid and passed through one and the same kaolin layer many times over. The filtrate was precipitated with 15 volumes of glacial acetic acid.
- 8) The substance recovered in the former treatment was taken up in 20 cc. of 1% hydrochloric acid, and after addition of 0.5 cc. of 20% phosphotungstic acid solution (no precipitation occurred), stirred up with kaolin and filtered. 10 volumes of glacial acetic acid were used for precipitation (fibrous precipitates).
- 9) Masamune, Suzuki and Kondoh, J. Bioch., 1940, **31**, 343.