Do Cancerous Ascitic Fluids Contain Mucoitin ?*

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Meyer and Chaffee¹⁾ isolated mucoitin (so-called hyaluronic acid) from the viscid pleural fluid of a patient with malignant tumors of the pleura and peritonium diagnosed as mesothelioma or endothelioma. According to the authors, the viscous factor of the fluid was this polysaccharide acid and had originated in tumor cells. They further imagined that the viscous carbohydrate, which Kabat²⁾ had obtained from fowl sarcoma and its cyst, is similar, if not identical, to mucoitin. It must be of significance to inquire as to whether or not mucoitin exists in the carcinomatous exsudate in serous cavities, because tumor cells here arise from the embryonal origin belonging to a different category.

Our material was a carcinomatous ascitic fluid from a patient with stomach cancer. It was submitted at first to dialysis and then to electrodialysis. After electrodialysis the insoluble part and the liquid in the middle compartment were separated. And the former was subdivided into a fraction insoluble in dilute alkali, that soluble in dilute alkali but insoluble in dilute acid and that soluble in dilute alkali as well as in acid. The latter was condensed and precipitated with alcohol. From each of these fractions a part was taken and treated with glacial acetic acid to remove phosphorus-containing substances. However, all the residues undissolved gave the negative naphthoresorcinol test for hexuronic acid, and were revealed by analysis to be simple proteins with comparatively small carbohydrate content. Thus it was proved that mucoitin was absent in the exsudate. To the same conclusion we came from the result of an attempt to separate mucoitin, if present, by means of formaldehyde. For this purpose were used fractions before treatment with glacial acetic acid and one of the final products above.

* 123rd report of Masamune and co-workers' Biochemical Studies on Carbohydrates.

EXPERIMENTAL.

Fractionation of the Ascitic Fluid.

870 cc. of a fairly viscid exsudate (pH 7.6) were drawn from the peritoneal cavity of a patient with diagnosis: carcinoma ventriculi with peritonitis carcinomatosa. The fluid was dialysed through cellophane against running water for 3 days and the content of the bag (the liquid (pH, 7.0) together with the voluminous precipitates, which had appeared) was subjected to electrodialysis (Water in the outer compartments was changed many times, until the pH of renewed portions was not shifted), and centrifuged. The centrifugate was washed with water, alcohol and ether, and dried in vacuo (Fr. P, 8.8 gm.). The supernatant liquid and water used for washing the centrifugate were combined and evaporated under reduced pressure, and the ropy condensate (transparent) dehydrated with alcohol, washed with alcohol and ether and dried (Fr. L, 6.1 gm.).

In the next, Fr. P was kneaded with some water, water increased to 180 cc. and dilute alkali (NaOH) dropped in cautiously to constant pH of 9.0. The viscous solution was separated from the insoluble part (Fr. P₁; this amounted to 0.25 gm. when dried) and acidified to pH 5.6 with dilute HCl, whereby precipitation occurred. The deposit was centrifuged, washed and dried as above (Fr. P₂; 4.129 gm.), and the mother liquid evaporated to syrupy consistency and precipitated with alcohol and washed and dried similar to above (Fr. P₃; 1.2 gm.). Since these fractions contained phosphorus (ammonium molybdate test), they were further treated with glacial acetic acid as follows. Substance was kneaded with a little water, taking the whole P₁ and 0.6 and 0.5 gm. of P₂ and P₃ respectively, diluted with drop by drop of glacial acetic acid at first and then glacial acetic acid further added as much as to precipitate all the part insoluble in it. The precipitate was washed with alcohol and ether and dried. On repeating this process twice, fractions free from phosphorus were obtained—Fr. P₁', 180 mgm.; Fr. P₂', 390 mgm.; Fr. P₃', 280 mgm. 0.6 gm. of L was also liberated likewise from phosphorus—Fr. L', 270 mgm.

Those products were all insoluble in water and, among them, P₁' and P₂' were insoluble also in dilute alkali and acid. P₃' was soluble in dilute alkali and mostly soluble in acid at pH 2.0, but precipitation occurred on lowering the pH of the acid solution. L' dissolved in alkali but did not in acid. All resembled one another qualitatively: negative ammonium molybdate (for phosphorus), aniline acetate (for pentose and hexuronic acid) and naphthoresorcinol (for hexuronic acid) tests; positive biuret, Molisch and Liebermann (for tryptophane) tests. Quantitative analysis gave the figures in Table I.

TABLE I.

Composition of the Non-electrodialyzable and Glacial Acetic Acid-insoluble Fractions of a Carcinomatous Ascitic Fluid.

(Figures express percentages.)

Fraction \ Analysis	N	Hexosamine	Sugar† as glucose by orcinol reaction	Ash
P ₁ '*	11.6	1.74	2.74	5.03
P ₂ '*	13.0	3.60	5.30	1.37
P ₃ '*	12.9	6.05	5.38	1.59
L'*	13.1	0.51	1.43	0.41

* Means the products in the text.

† Substance was hydrolysed for 3 hours with 1 n. H₂SO₄ in an ampoule (temp., 100°C) and the filtrate analysed. The colours developed by orcinol, diphenylamine and indole did not coincide in intensity, as expressed as any of hexoses (glucose, mannose and galactose).

A Search for Mucoitin by means of Formaldehyde.³⁾

1.5 gm. of Fr. P₂, 0.8 gm. of Fr. P₃, 3.0 gm. of Fr. L and 0.05 gm. of Fr. P₁', which were left unused in the experiment above (in total, 5.35 gm.), were suspended together in 100 cc. of water and alkalized with 1% Na₂CO₃ to pH 8.4, whereby almost all went into solution. After addition of 10 cc. of formalin and bringing back the lowered pH to 7.8, the mixture was made up to the volume of 200 cc. with water. Then it was shaken on a machine for 24 hours (pH at this stage, 7.6) and centrifuged. The supernatant liquid and the washings of the centrifugate combined were acidified to pH 4.6 (30% acetic acid used). The voluminous precipitate, that came out, was centrifuged and washed with two 20 cc. portions of dilute acetic acid of the same pH and the supernatant liquid and the washings were united and precipitated with 4 volumes of 95% alcohol and anhydrous sodium acetate. This precipitate was washed with alcohol repeatedly, with addition of anhydrous sodium acetate when necessary, and dried in a desiccator. 250 mgm. of brownish substance were obtained.

The product above was extracted exhaustively with water. The extracts (some were slightly turbid) were evaporated together under diminished pressure to about 8 cc., and 40 cc. of absolute alcohol and 0.2 gm. of anhydrous sodium acetate added. The precipitate was separated, washed with alcohol and ether and dried. It amounted to 180 mgm. On re-extractions of it and evaporation and precipitation of the extracts, the amount of substance decreased to 50 mgm. Its naphthoresorcinol test of Neuberg and Saneyoshi for hexuronic acid resulted in negative. Biuret

and Molisch reactions were given and phosphorus was detected.

To remove phosphorus the substance was treated with glacial acetic acid. Namely, it was kneaded with 2 drops of water, glacial acetic acid added at first in drops and afterwards in larger portions (in total, 15 cc.) and finally anhydrous sodium acetate (0.3 gm.) added to complete the precipitation. The centrifugate was washed with alcohol many times and with ether twice and dried. This procedure with glacial acetic acid and anhydrous sodium acetate was effected twice more, using here ether together because of insufficient precipitation by the former agents alone. Yield, 27 mgm. This phosphorus-free substance also gave neither Goldschmiedt reaction with α -naphthol nor Neuberg-Saneyoshi test with naphthoresorcinol nor aniline acetate reaction. Biuret and Molisch reactions were positive.

To note in passing, the product at the end was a mixture of a water-insoluble, a water-soluble but acid(pH, 3.8)-insoluble and a water- and acid-soluble substance, the last of which was biuret- and Molisch-positive and gave the direct reaction of Osaki and Turumi but not the indirect.

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Bibliography and Notes.

- 1) Karl Meyer and Chaffee, J. Biol. Chem., 1940, **133**, 83.
 - 2) Kabat, *ibid.*, 1939, **130**, 143.
 - 3) Compare with the separation method of mucoitin by Osaki and Hasegawa, Tohoku J. Exp. Med., 1943, **45**, 176.
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