Fractionation of Urinary Low Molecular Weight Carbohydrate-Containing Compounds

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Increasing attention has recently been paid to the urinary mucosubstances of low molecular weight.[1-5] The compounds of this type isolated so far contain hexoses, hexosamines, and amino acids but appear to be poor in neuraminic acid.[1,3,5] However, fractionation of either native urine [6] or its ultrafiltrate [7] by gel filtration, followed by paper electrophoresis of one of the subfractions so isolated,[8] demonstrates the presence of several neuraminic acid-containing compounds. In the present paper preliminary results are reported concerning the further fractionation of dialysable carbohydrate-containing material, separated from urine by gel filtration, on either an anion exchange or an activated carbon-Celite column.

Anion exchange chromatography. Concentrated urine filtered through a Sephadex G-25 column (2.1 × 65 cm) gave, as previously reported,[6,8] peaks A, B, and C for neuraminic acid. Peak A, eluted directly from the column, contained non-dialysable material. This was followed by peak B, which contained about 30 % of the dialysable neuraminic acid. The rest, 70 %, was eluted in peak C.

A column of Dowex-2X 8 (0.6 × 8 cm) in borate form was prepared according to Masamune *et al.*[2] Peak B of the gel filtration was pooled, dried *in vacuo*, dissolved in a small amount of 0.005 M borate-HCl buffer (pH 8.6) and applied on to the column. Elution was performed stepwise with 5 ml portions, as indicated in Fig. 1. The eluate was analysed for hexose (including fucose), hexosamine, sialic acid, glucuronic acid and Folin-positive material by the same methods as in earlier studies.[8] The ion exchange purification on Dowex-50 [9] was employed in hexosamine assays, since borate reduces the colour intensity of the Elson-Morgan reaction.[10] Fig. 1 indicates that six different subfractions are obtained from the material present in peak B with the elution system used. According to the colour reactions, all but the first

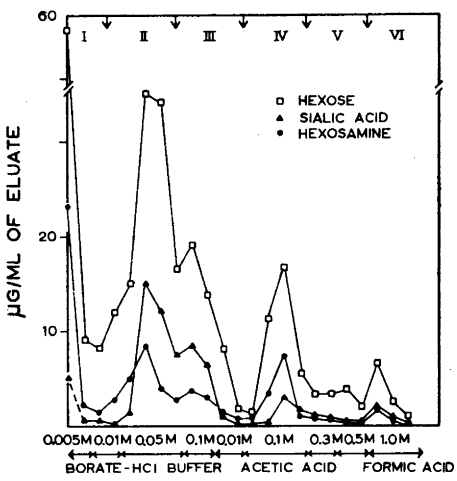

Fig. 1. Elution curves for hexose, hexosamine and neuraminic acid obtained by subjecting the material of peak B of the urinary gel filtration (see text) to chromatography on an anion exchange column. Stepwise elution was performed with borate-HCl buffer (pH 8.6), acetic acid and formic acid as presented on the abscissa. Subfractions, designated I–VI, were pooled for paper chromatography as indicated by arrows.

one contain neuraminic acid and traces of uronic acid. The Folin-positive material of peak B was eluted almost entirely with fraction IV. Paper chromatography was employed in the identification of the monosaccharide components present in each fraction. Salts, mainly borate and chloride, were removed with a short carbon-Celite column. This was possible because the material present in fraction B is almost completely retained by charcoal on the one hand and can be eluted from the column on the other hand (see later). Material so obtained was hydrolysed with 1 M HCl at 100° for 1 h and subjected to paper chromatography (ethyl acetate:pyridine:water, 10:4:3). Aniline oxalate was used as indicator. No attempts were made to detect neuraminic acid. The results showed that glucose and galactose were present in each fraction. Spots for mannose, fucose, and aminosugars were seen at least in fractions I–IV and for glucuronic acid in fractions IV and VI. At least two amino acids were present in each fraction as

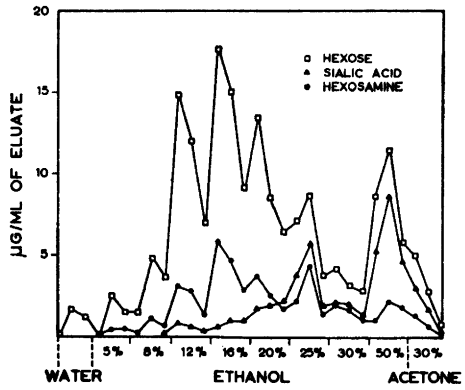

Fig. 2. Elution curves for hexose, hexosamine and neuraminic acid obtained by subjecting the same material as in Fig. 1 to chromatography on an activated carbon-Celite column. Elution was performed with water, ethanol, and acetone as indicated on the abscissa.

judged on the basis of one-dimensional chromatography on thin-layer plates (Kieselgel G; phenol:water, 75:25).[11] The fractions were desalted as above and hydrolysed extensively in 4 M HCl.

Chromatography on activated carbon. Both the broadness of the peaks and the varying relation of the different carbohydrates to each other within the peaks indicate that the fractions in Fig. 1 are still quite heterogeneous. Further evidence for this is presented in Fig. 2, which shows that an entirely different pattern is obtained when the material present in peak B is subjected to chromatography on activated carbon. This was performed by applying the pooled and concentrated material of peak B on a small charcoal-Celite column (0.6 × 8 cm; Darco G—60: Celite 545, 1:1, w:w) and eluting with 5 ml portions of water, ethanol, and acetone as presented in Fig. 2. The compounds which were eluted with 5—20 % ethanol were rich in hexose and hexosamine, whereas those eluted with 25—50 % ethanol and 30 % acetone were rich in neuraminic acid.

Chromatographies carried out with the material present in peak C of the gel filtration on both anion exchange and charcoal columns indicated that even there several components differing in carbohydrate composition are present.

1. Hakomori, S., Kawauchi, H. and Ishimoda, T. *Biochim. Biophys. Acta* **65** (1962) 546.
2. Masamune, H., Hakomori, S. and Sugo, T. *Tohoku J. Exptl. Med.* **69** (1959) 383.
3. Hakomori, S. and Ishimoda, T. *J. Biochem. (Tokyo)* **52** (1962) 250.
4. Brada, Z. *Nature* **197** (1963) 592.
5. Pechan, Z. *J. Chromatog.* **10** (1963) 104.
6. Miettinen, T. A. *Scand. J. Clin. Lab. Invest.* **14** (1962) 380.
7. Lundblad, A. and Berggård, I. *Biochim. Biophys. Acta* **57** (1962) 129.
8. Miettinen, T. A. *Clin. Chim. Acta* **8** (1963) 693.
9. Boas, N. F. *J. Biol. Chem.* **204** (1953) 553.
10. Tracey, M. V. *Biochim. Biophys. Acta* **17** (1955) 159.
11. Brenner, M. and Niederwieser, A. *Experientia* **16** (1960) 378.

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Correction to "A New Vanadium Monosulfide" *

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On p. 2353, paragraph 6, line 7, the value of the z parameter for sulfur is incorrectly stated. It should read $z=0.926$.

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