

## The isolation of sialic acid in high yield from colostrum

The wide distribution of sialic or neuraminic acids throughout the animal kingdom has rightly made them the object of considerable recent study. Several methods of isolation of certain derivatives of neuraminic acid from various biological materials have been reported. The preparation of neuraminic acid has recently been well reviewed by ZILLIKEN<sup>1</sup>. Two materials rich in sialic acid are cow colostrum and human milk, analyzing as high as a gram and more per liter. Because of the low yields obtained, isolation from these fluids necessitates starting with large volumes (20 l) to obtain reasonable quantities of product<sup>2,3</sup>. These procedures generally involve a dialysis step to remove lactose and inorganic salts. This technique results in considerable loss of sialic acid, particularly in the case of colostrum where a substantial portion of this acid is present in the dialyzable form.

The method described in this paper for the isolation of sialic acid from colostrum has the advantages that it requires no dialysis step, is simple to carry out, and that a starting volume of 1 l is ample to prepare 0.5 g or more of crystallized material. The procedure involves six principal steps: acid hydrolysis of the colostrum, removal of the protein by filtration, separation of the sialic acid by ion exchange, elution of the product with dilute acid, lyophilization and crystallization. The techniques employed in hydrolysis and ion exchange are essentially those used by ZILLIKEN<sup>2</sup> in his isolation procedure using human milk. The sialic acid from colostrum has been identified as *N*-acetylneuraminic acid.

Colostrum, from the first 24 h of milking after parturition, was defatted at 32° using a small cream separator. 1 l of the defatted material was adjusted to a pH of about 1.1-1.3 using 6 *N* H<sub>2</sub>SO<sub>4</sub>, and heated at 80-85° for 2 h. The mixture, after cooling, was filtered, and the protein precipitate pressed dry on a Buchner funnel. The cake was twice slurried with about 250 ml of water, filtered and pressed dry. The pooled filtrate and washings were neutralized with solid BaCO<sub>3</sub>, and filtered. This precipitate, too, was washed twice with 100-ml portions of water.

The combined filtrate and washings were put through an Amberlite IR-120 (H<sup>+</sup>) column (35 × 400 mm), and washed through with 300 ml of water. The effluent was in turn put through a Dowex-1 X8 column (29 × 450 mm), formate form, 200-400 mesh. The column was washed with water until the Molisch test for sugars was completely negative. It was then eluted with formic acid, using the gradient-elution technique.

The eluate was collected in 10-ml aliquots. Those tubes giving both a positive BIAL<sup>4</sup> and WARREN<sup>5</sup> thiobarbituric acid reaction were combined, shell frozen, and lyophilized to yield a white to light-tan powder. The powder was dissolved in the minimum possible volume of water, and glacial acetic acid added in the amount of 5.0 ml/ml of water used. After allowing crystallization to proceed overnight, the product was filtered off, washed with cold acetic acid, then ether, and air dried to give 400-600 mg of material. This product could be further crystallized, with some loss, from BLIX's solvent system<sup>6</sup>.

The analysis for a sample of product, dried *in vacuo* at room temperature over P<sub>2</sub>O<sub>5</sub> and KOH follows: Found: C, 42.88; H, 6.28; N, 4.45; ash, nil. Calc. for C<sub>11</sub>H<sub>19</sub>O<sub>9</sub>N: C, 42.72; H, 6.19; N, 4.53%.

The infrared spectrogram of a typical preparation, run in compressed KBr, is

given in Fig. 1. Also shown is the pattern for a sample of synthetic *N*-acetylneuraminic acid prepared according to the method of CORNFORTH *et al.*<sup>7</sup> The two spectrograms are essentially identical. The product was laevorotatory, with an  $[\alpha]_D^{25}$  of  $-31.6^\circ$  as determined on a 1% solution in the Standard Model D Keston Photoelectric Polarimeter\*. The material moved as a single spot when chromatogrammed

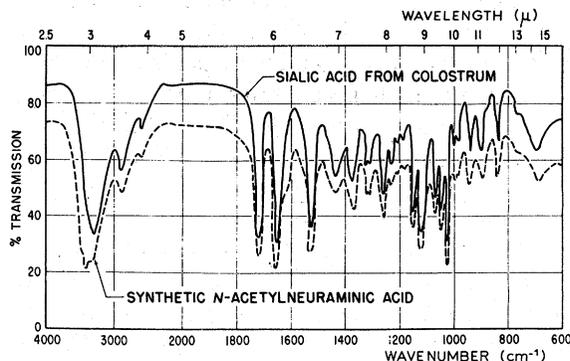


Fig. 1. Infrared patterns of sialic acid from colostrum and synthetic *N*-acetylneuraminic acid.

on Whatman No. 1 filter paper using butanol – pyridine – water (6:4:3), and developed with the thiobarbituric acid reagent of WARREN<sup>8</sup>. The  $R_F$  for this system was 0.08, and the material moved identically to an authentic sample of *N*-acetylneuraminic acid. When the solvent system of butanol – acetic acid – water (50:12:25) was used, the product gave a principal spot with an  $R_F$  of 0.22 with a very faint trailing spot,  $R_F$  0.15.

Filtration of the colostrum hydrolysate, both before and after neutralization, is necessary. It effectively removes nearly all of the protein. However, material precipitated by trichloroacetic acid is definitely present in the final filtrate. This material apparently passes through both the Amberlite and Dowex resins without adsorption, as indicated by a trichloroacetic acid test of the effluents. Both the effluent and the wash from the Dowex column gave a completely negative test in the BIAL and WARREN thiobarbituric acid reaction.

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<sup>1</sup> M. W. WHITEHOUSE AND F. ZILLIKEN, *Methods of Biochem. Anal.*, 8 (1960) 199.

<sup>2</sup> F. ZILLIKEN AND P. J. O'BRIEN, *Biochem. Preparations*, 7 (1960) 1.

<sup>3</sup> F. ZILLIKEN, G. A. BRAUN AND P. GYÖRGY, *Arch. Biochem. Biophys.*, 63 (1956) 394.

<sup>4</sup> N. M. PAPADOPOULOS AND W. C. HESS, *Arch. Biochem. Biophys.*, 88 (1960) 167.

<sup>5</sup> L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.

<sup>6</sup> G. BLIX, E. LINDBERG, L. ODIN AND I. WERNER, *Acta Soc. Med. Upsaliensis*, 61 (1956) 1.

<sup>7</sup> J. W. CORNFORTH, M. E. FIRTH AND A. GOTTSCHALK, *Biochem. J.*, 68 (1958) 57.

<sup>8</sup> L. WARREN, *Nature*, 186 (1960) 237.

\* The mention of trade names in this paper is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U.S. Department of Agriculture.