The work described above confirms HAMPTON's observation that polio virus is dissociated by lyophilization. Our observations, however, differ from HAMPTON's in that we have found that the virus particles do not break up into units of uniform size, but that a variety of particles of different dimensions were formed. From results obtained in the ultracentrifuge it would appear that two main components are formed as products of the dissociation and have sedimentation coefficients of 66 to 69 S and approximately 15 S, respectively. It cannot be decided at the moment whether the faster of these components consists of the intact nucleic acid nucleus or the protein shell or some bigger fragments of either part of the virus. The slower component undoubtedly consists of smaller dissociation products with molecular weights of probably less than a million. As only a trace of the lyophilized preparations was insoluble and only 40–60 % was recovered as sedimented material, much must have been converted to even smaller particles of sedimentation coefficients less than 15 S.

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*Biochemical Department, Nobel Medical Institute, Stockholm (Sweden)* A. EHRENBERG
*C.S.I.R. and U.C.T. Virus Research Unit, Department of Pathology, University of Cape Town (South Africa)* A. POLSON

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## The sialic acids of hog pancreas

The amino group of sialic acids can be substituted with an acetyl group (N-acetylsialic acid) or a glycolyl group (N-glycolylsialic acid). BLIX *et al.*[1] isolated the glycolyl-containing sialic acid (P-sialic acid) from hog-submaxillary mucin. When we tried to isolate N-glycolylsialic acid from other tissues of hog, *e.g.* kidney and serum, the crystals were found to be composed of about 15 % N-glycolylsialic acid and 85 % N-acetylsialic acid[2,3]. On the other hand, the sialic acids isolated by us from the submaxillary gland and mucin of hog contained 80 % and 90 % N-glycolylsialic acid, respectively. Therefore, we assumed that the mucoids in the secretions from the hog glands contained practically pure N-glycolylsialic acid. To test the hypothesis we have isolated the sialic acids from hog pancreas and investigated their composition.

2 kg of hog pancreas freed from most of the fat and connective tissue were homogenized in a Turmix blendor. The homogenate was poured under stirring into 8 l ethanol and boiled for 30 min. The ethanol was filtered off and the residue was reboiled with the same volume of ethanol. The extracted material was collected on a Büchner funnel and washed several times with ethanol and finally suctioned dry. The washing of the material and the isolation of sialic acids were carried out as described for human liver[4]. The lyophilized crude fraction contained 315 mg sialic acid determined by the resorcinol method[5]. As the amount of N-acetylsialic acid in the original material was found to be 725 mg, the yield was 43 %.

Lyophilized material, corresponding to 275 mg sialic acids, was dissolved in 2 ml water and diluted with 20 ml methanol. 50 ml diethyl ether were added under continuous agitation. A large amorphous precipitate was filtered off and an additional 10 ml of ether added. Crystallization started immediately. The crystals were redissolved in water and methanol, and ether was added as before. The yield of recrystallized sialic acids was 137 mg (Fracion A).

The amorphous precipitate and the mother liquors were rechromatographed on Dowex-2. A second fraction of sialic acids could be crystallized (Fraction B = 61 mg).

Table I shows the glycolic acid content of the lyophilized material and of Fractions A and B.

Only two spots were found, with the same $R_F$ values as N-acetylsialic acid and N-glycolylsialic acid, when the material was subjected to paper chromatography with *n*-butanol–*n*-propanol–0.1 NHCl (1 : 2 : 1, v/v/v) as solvent[7].

The X-ray powder diagram was identical with that of P-sialic acid[1,2] (N-glycolylsialic acid). The infrared spectrum was of the same type as that of a mixture of the two sialic acids[7].

TABLE I

GLYCOLIC ACID CONTENT OF FRACTIONS ISOLATED FROM HOG PANCREAS

| | *Glycolic acid (%)* | *N-Glycolylsialic acid (%)* |
|---|---|---|
| Lyophilized material | 14.0 | 60 |
| Fraction A | 14.6 | 62 |
| Fraction B | 15.0 | 64 |

Glycolic acid determined by the procedure of KLENK AND UHLENBRUCK[6].

The results are given as percentages of the sialic acid content determined by the resorcinol reaction.

Since the yield of the sialic acids was rather low, enrichment of one type of sialic acid must be considered. However, the liberation of the two sialic acids went parallel, as judged from the paper-partition chromatograms, and during the crystallization of the sialic acids, no significant change in the ratio of the two sialic acids occurred (see Table I).

The present results indicate that the mucoids of the secretory glands in the hog have a much higher percentage of N-glycolylsialic acid than the glycoproteins of the serum-protein type. The percentage of the glycolyl-containing sialic acid was lower in the pancreas than in the submaxillary gland. The pancreas secretion is, however, more serous, and the mucoid concentration is rather low in comparison with that in the submaxillary gland. As the amount of glycoproteins of the serum type probably is the same in the two organs, a lower glycolyl content in the sialic acids of hog pancreas is to be expected if the hypothesis is valid.

SVEN-OVE PETTERSSON
RAMON SIVERTSSON
STELLAN SJÖGREN
LARS SVENNERHOLM

*Department of Medical Biochemistry, University of Gothenburg, Gothenburg (Sweden)*

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## An effect of drying the isolated cell walls of *Streptococcus faecalis* and a *Pseudomonas* species

Extraction of acetone-dried bacterial cells with cold 5% TCA* is a method in general use for obtaining preparations of surface polysaccharides and related complexes. In an investigation to be reported elsewhere, cytoplasmic constituents of *Streptococcus faecalis* (N.C.T.C. No. 6782) and a species of *Pseudomonas* (described previously[1]) were being examined in TCA extracts of acetone powders and it became necessary to learn whether or not any cell-wall components might appear in the extracts. It seemed probable that a surface material, analogous to the so-called "O" somatic antigen of other Gram-negative bacteria, was being removed from the *Pseudomonas* but the strain of *S. faecalis* was not known to produce any such component. In the Gram-negative bacteria the relationship between the surface polysaccharides and the structural components of the rigid cell wall is uncertain and the effect of TCA on the isolated cell walls of both Gram-negative and Gram-positive bacteria has not been investigated.

Cell walls were prepared from stationary-phase cells by Mickle disintegration[2] followed by tryptic digestion, washing and, where applicable, freeze-drying. Cell-wall preparations (15–50 mg) were extracted overnight at 3° with TCA (5% w/v, 20 ml) or water (20 ml) and centrifuged off. TCA-extracted cell walls were washed once with cold 5% TCA (20 ml), 3 times with water and freeze-dried. Water-extracted cell walls were washed once with cold water (20 ml) and freeze-dried. Monosaccharide constituents of the various preparations were detected after hydrolysis for 2 h at 100° with 2 *N* HCl by paper chromatography in butan-1-ol–acetic acid–water, 6:1:2 and butan-1-ol–ethanol–water, 5:1:4 (top layer) and reaction with *p*-anisidine hydrochloride or

* Abbreviations used are; DAP, *α,ε*-diaminopimelic acid; DNP-, 2,4-dinitrophenyl-; FDNB, 1-fluoro-2,4-dinitrobenzene; TCA, trichloroacetic acid.