The Sialic Acids of Hog Gastric Mucosa

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The mucoids and the mucopolysaccharides of hog gastric mucosa have been subject to great interest because they contain compounds of physiological importance as, *e. g.*, the intrinsic factor and the bifidus factor. Several carbohydrate components have been isolated from the gastric mucosa, but hitherto not the sialic acids, although Werner and Blix [1] have determined their amount in gastric mucosa to 3—5 %. In previous papers [2-4] it has been shown that the composition of the sialic acids in hog material showed large variations. In the pancreas and the submaxillary gland N-glycolylsialic acid was the main component, but in the serum proteins N-glycolylsialic acid constituted only 10—15 % of the total sialic acids. Therefore, we postulated that in the epithelial mucins N-glycolylsialic acid occurred practically pure but in the glycoproteins of serum type N-acetylsialic acid was the dominating acid.

Experimental. Isolation of sialic acids. Fifty stomachs from hogs were collected immediately after slaughter and washed free from food contaminants and floating mucus. The mucosal linings of the corpus and the canalis were scraped off — yield 2.3 kg — and poured into 10 l of ethanol. The mixture was brought to boiling. The ethanol was filtered off and the residue was treated with ethanol once more. The residue was then treated with weak sulphuric acid and the sialic acids isolated as described for human liver [5]. The yield of lyophilized sialic acids was 580 mg which constituted about 80 % of the calculated value. The sialic acids were crystallized from a methanol-water solution by adding diethyl ether. Yield 534 mg (Fraction A).

As the ethanol solution also gave a positive sialic acid reaction it was concentrated *in vacuo* to about 1 l. It was shaken three times with chloroform in a separatory funnel for removing the lipids. After filtration the lipid-free solution was run through Dowex 50 (H^+) and then on a column with Dowex 1 in hydroxyl-formate (9:1, v/v) form. (Because of the large amount of salt the sialic acids were not attached to the anion exchange resin when it was in acetate or formate form.) The sialic acids were eluted from the resin with 0.3 N formic acid as earlier described [5]. Yield 110 mg. The yield of crystallized material was only 56 mg due to an accident at the crystallization (Fraction B).

Results. Elementary analysis. Fraction A: C 42.37; H 6.22; N 4.42. Calc. for N-acetylsialic acid ($C_{11}H_{19}NO_9$): C 42.72; H 6.19; N 4.53. Calc. for N-glycolylsialic acid ($C_{11}H_{19}NO_{10}$): C 40.61; H 5.89; N 4.31. *Glycolic acid* was determined with the method of Klenk and Uhlenbruck [6] with minor modifications [3]. Fraction A: 4.5 % glycolic acid, corresponding to 19 % N-glycolylsialic acid. Fraction B: 5.8 % glycolic acid, corresponding to 25 % N-glycolylsialic acid. *Optical rotation:* Fraction A: $[\alpha]_D^{20} = -30.8°$.

Paper partition chromatography: As solvent was used *n*-butanol-*n*-propanol-0.1 N hydrochloric acid (1:2:1, v/v). Two spots were indicated with the same position as N-acetylsialic acid and N-glycolylsialic acid. The *X-ray powder diagrams* were of the O-sialic acid (N-acetylsialic acid) type [7]. The *infrared spectrum* was of the same type as that of a mixture of the two sialic acids.

Discussion and summary. The sialic acids from hog gastric mucosa have been isolated and crystallized. They were obtained in a good yield and were shown to be a solid solution of 20 % N-glycolylsialic acid in 80 % N-acetylsialic acid. The ratio N-glycolylsialic acid/N-acetylsialic acid is somewhat greater than in the serum proteins but far from the ratio in the submaxillary mucin. If the hypothesis is valid that the mucoids of the mucous secretions of hog is practically pure N-glycolylsialic acid, the sialic acids in gastric mucosa are mainly derived from proteins of serum type.

The great amount of free sialic acids is remarkable as they have not been found earlier in free form in biological materials. The sialic acids were not liberated during the isolation by the boiling ethanol, as free sialic acids were indicated also at the precipitation of the mucosa with ethanol in the cold. They were probably liberated by the hydrochloric acid in the stomach but no test was made for sialidase. Work is now in progress to analyse the concentration of free sialic acids in the gastric juice from normal and achlorhydric subjects.

Recently it was described that some carbohydrates enhanced the vitamin B_{12} absorption [8]. It is possible that the free sialic acids may be of importance for this transport.

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The Losses of Vitamin A during Chromatography

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When the irrelevant absorption in the spectrophotometric determination of vitamin A exceeds certain limits, the geometric correction method according to Morton and Stubbs [1] is unreliable, and purification of the vitamin A fraction by chromatography is recommended. Actually chromatography is included in the procedure described in official methods in some countries. The chromatography is most often carried out on alumina columns by some modification of the method described by Gridgeman, Gibson and Savage [2] for the estimation of vitamin A in whale liver oils. A certain activity of the alumina is needed to obtain a sufficient separation of the unsaponifiable substances, but unfortunately a higher activity also means a higher loss of vitamin A in the procedure. In our experience the most potent oils give the highest losses, even up to 10 % for some vitamin A concentrates. Such losses can be counteracted by deactivation of the alumina. Usually a standardized activity is obtained by uniform water addition. To avoid this difficulty the use of a softer adsorbent, *e.g.* calcium phosphate has been introduced [3]. It is, however, of importance to obtain standardized columns in different laboratories, and alumina is the adsorbent preferred as it can be obtained readily in uniform quality everywhere as "Aluminium Oxide Standardized for Adsorption analysis according to Brockman". As mentioned above, a softening of the alumina is necessary to make it useful. Such deactivation will, however, result in a reduced separation of the components in the unsaponifiable matter. A limit thus has to be found where conditions are optimal all factors considered. This means that a certain loss is unavoidable, and must be controlled and taken into consideration by using a standard.

We have been trying to find substances which, added to the vitamin A fraction, would reduce the losses during the chromatography. Gridgeman *et al.*[2a] found it advisable to add cholesterol to make up the weight of the unsaponifiable matter if this amounted to less than 0.35 g. We found cholesterol to be of no effect in preventing the loss of vitamin A. Neither did several other types of substances, *e.g.* fatty alcohols or glyceryl ethers, have any such effect. The unsaponifiable matter of ground nut oil, however, gave a striking reduction in the loss. In one experiment the unsaponifiable matter of 0.5 g of ground nut oil gave a recovery of 98 % of a total of 2 000 I.U. vitamin A compared to 90 % without addition. The effect was found in the fraction of the unsaponifiable matter passing the alumina column with a speed similar to vitamin A. Analysis on paraffinated paper as described by Green, Marcinkiewicz and Watt [4] showed that the effective fraction contained γ-tocopherol. Pure γ-tocopherol (Eastman-Kodak) was then tried, and the effect was obtained with as little as 200 μg added per 1 000 I.U. vitamin A put through the column. α-Tocopherol had no effect, but as other slow moving tocopherols might be effective, the unsaponifiable matter of other vegetable oils were tried. Soy bean oil with its high concentration of γ- and δ-tocopherols (1.000 μg per g compared with 200 μg per g in ground nut oil) protected effectively against losses. Alumina columns of the