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Short Communications

Protein-bound Sialic Acids in Human, Hog and Horse Kidneys

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As part of an investigation of the protein-bound carbohydrates in mammalian kidneys, carried out by one of us (E.M.), the sialic acids were isolated from human, hog and horse kidneys. Blix *et al.*[1] have by the X-ray diagram pattern differentiated between four sialic acids, *viz.* ovine (N-acetylsialic acid), porcine (N-glycolylsialic acid), bovine and equine (N, O-diacetylsialic acid) sialic acids. All these acids were isolated from the submaxillary mucin of the animals. The procedure applied by Blix *et al.* is unsuitable for the isolation of sialic acids from organs with a low sialic acid content[2]. In such cases chromatography on anion exchange resins is to be preferred, but by this technique only N-acetylsialic acid and N-glycolylsialic acid can be isolated quantitatively as the labile O-acetyl group is partly split off in the course of the preparation. However, our main problem was to ascertain the possible occurrence of N-glycolylsialic acid, as at quantitative estimation it gives about 20 % higher molar absorbancy than N-acetylsialic acid and N,O-diacetylsialic acid[3,4]. The three species investigated were chosen because their submaxillary mucins contain different sialic acids.

Experimental. Materials. Human kidneys, from three young healthy persons killed by accidents, were removed as soon as possible after death (12—24 h) with intact blood vessels. The hog and horse kidneys were also dissected with intact vessels.

Isolation procedure. The kidneys were perfused with physiological saline through their renal artery until the perfusion solution was free from visible amounts of blood cells or pigments. Then the kidneys were homogenized in a Turmix blendor and extracted twice with four volumes of acetone at +4°C for 24 h. The remaining lipids were extracted with methanol-chloroform (2:1 v/v) in a Soxhlet apparatus for 24 h. The isolation of the sialic acids from the defatted kidney tissues was performed with the ion-exchange procedure earlier described[2]. The lyophilized sialic acids were rather impure and rechromatography on the resins was done as a routine. The yields of recrystallized sialic acids were 50—75 % (the lowest yield was obtained from horse kidney).

Identification. The specific optical rotation had the same value for the three specimens, $[\alpha]_D$ —31° ± 1. In paper partition chromatography[2] an elongated spot was given by hog and horse sialic acids, while the spot of the human acid was more round and distinct. The X-ray diffraction patterns (Fig. 1) of the sialic acids were all of the same type as the pattern of ovine sialic acid[1] (N-acetylsialic acid). No lines of the porcine sialic acid pattern were indicated. The determination of glycolic acid, performed according to Klenk and Uhlenbruck[5], showed that the human sialic acid contained no glycolic acid, while the other two contained about 3 % of glycolic acid (Table 1).

Discussion. Although the X-ray diffraction patterns only showed the occurrence of N-acetylsialic acid, the acids from hog

Table 1. Determination of glycolic acid in sialic acids.

Source of sialic acid	Glycolic acid, %	Calc. amount of N-glycolylsialic acid, %
Human kidney	0.1	0
Hog kidney	3.2	14
Horse kidney	2.6	11
Human serum	0.1	0
Hog submaxillary mucin	20.9	90

Fig. 1. X-Ray diffraction patterns of sialic acids isolated from (a) human, (b) hog and (c) horse kidneys.

and horse kidneys contained appreciable amounts of glycolic acid. The diversity of the results might be explained either by some unspecificity of the glycolic acid method or by lacking sensitivity of the X-ray method for rather large admixtures of a second sialic acid.

The first alternative seems rather unlikely as no glycolic acid could be indicated in sialic acids from human sources. The sialic acid isolated from hog submaxillary mucin contained 90 % of the theoretical value for N-glycolylsialic acid. In the resorcinol method [4] the sialic acids from hog and horse kidneys had some per cent higher molar absorbancy indices than our standard sample of N-acetylsialic acid. That is also a support of the occurrence of N-glycolylsialic acid, as the latter has about 20 % greater molar absorbancy index than N-acetylsialic acid.

Regarding the second alternative it might be possible that N-glycolylsialic acid is built into the crystal lattice of N-acetylsialic acid, as the two sialic acids only differ by the substitution of a hydrogen atom in the acetic acid moiety of N-acetylsialic acid with a hydroxyl group. We have also evidence that N-acetylsialic acid can be built into the crystal lattice of N-glycolylsialic acid. In a preparation of sialic acid from beef serum N-acetylsialic acid made out 60 % and N-glycolylsialic acid 40 %. The X-ray diffraction pattern was the same as that of porcine sialic acid (N-glycolylsialic acid). This may appear puzzling as the amount of N-glycolylsialic acid is lower than that of N-acetylsialic acid, but might be explained by the lower solubility of N-glycolylsialic acid.

Conclusion. The sialic acids from human, hog and horse kidneys were isolated. X-Ray diffraction patterns showed N-acetylsialic acid only, but the estimation of glycolic acid demonstrated that hog and horse sialic acids also contained N-glycolylsialic acid. It is concluded that the X-ray diffraction pattern is unsuitable as a criterion of the uniformity of a sialic acid.

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