## Note

# The type and content of the sialic acid of bile from several animal sources*

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In contrast to such other glycoproteins as bovine and ovine submaxillary mucins, the bile mucins have been the object of only limited investigations[1–6]. This may be due to the scarce knowledge of the bile composition The occurrence of several sialic acids in the gallbladder of calf was reported[7] in 1965 It was found that *N*-glycolylneuraminic acid was accompanied by other acylneuraminic acids, principally *N*-acetylneuraminic acid The present Note describes the identification and quantitative determination of protein-bound sialic acids from the bile of several mammalian species

## MATERIALS AND METHODS

Except for the human species, various vesicular biles were the source 7500 ml from 21 bladders of adult cows, 7100 ml from 25 bladders of adult pigs, 300 ml from 38 bladders of lambs, and 400 ml from several samples of vesicular and hepatic human bile were used The glycoprotein fraction was obtained by ethanol precipitation[7] The bile was added to four times its volume of cold 96% ethanol After 8 h, the mixture was boiled for 30 min and then decanted, two volumes of 96% ethanol were added to the precipitate and the mixture was boiled again for 30 min The cooled precipitate was separated and washed with 5mM sulfuric acid, several times at 4°, until a pH of 2 5 was reached The precipitate was hydrolyzed with 50mM sulfuric acid, for 45 min, at 80°

The separation and purification of the acylneuraminic acids were performed according to Klenk and Uhlenbruck[8], as previously described[7 9]. The qualitative and quantitative analyses of the sialic acids were carried out by paper chromatography with the following solvents (a) butyl alcohol–acetic acid–water (4 1 5, v/v), (b) butyl alcohol–propyl alcohol–0 1M hydrochloric acid (1 2 1, v/v), (c) butyl alcohol–pyridine–0 1M hydrochloric acid (5 3 2, v/v); and (d) ethyl acetate–acetic acid–water (3 1 3, v/v) The colorimetric procedures were reported in previous publications[7 9 10] The modified Ehrlich spray[11] was generally employed for staining

*Dedicated to Professor J -É Courtois in honor of his 65th birthday

chromatograms; quantitative determinations were always performed by the modified resorcinol procedure[12,13] or the thiobarbituric acid method[14,15], in some cases, the Eegriwe test was used to determine *N*-glycolylneuraminic acid[8].

TABLE I

SIALIC ACIDS FROM BILE GLYCOPROTEINS

| *Sialic acid* | *Purified glycoproteins*[a] | | | |
|---|---|---|---|---|
| | Bovine | Porcine | Ovine | Human |
| *N*-Glycolylneuraminic acid | 10 | 13 | 15 | |
| *N*-Acetylneuraminic acid | 23 | 48 | 64 | 70 |
| *N,O*-Diacetylneuraminic acid | 67 | 39 | 7 | 30 |
| Sialic acid-containing oligosaccharides | | | 14 | |

[a]Proportion of sialic acids in per cent.

## RESULTS AND DISCUSSION

Elution of the acylneuraminic acids from anionic columns produced one peak in all cases Crystallization of these acids produced the typical needle forms Table I summarizes the main results on the nature and content of each sialic acid in the purified glycoproteins A positive thiobarbituric acid test indicates that the *N,O*-diacetylneuraminic acid obtained is probably *N*-acetyl-4-*O*-acetylneuraminic acid, on the basis of the specificity of this reaction[14–16] The sialic acid-containing oligosaccharide is possibly a disaccharide, on the basis of its $R_I$ and staining properties

It may be concluded that the sialic acids isolated from human, bovine, ovine, and porcine bile glycoproteins differ both in their nature and relative concentration In all cases *N*-acetylneuraminic acid is present, but human bile does not contain *N*-glycolyneuraminic acid

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