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Bile Glycoproteins from Several Species

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In the bile glycoproteins of the abovementioned species the following hexoses and hexosamines were generally found: D-galactose, D-mannose, and L-fucose, D-glucosamine and D-galactosamine, but their concentrations differend remarkably. The common amino acids were found; the amounts of aspartic and glutamic acids, leucine, glycine and valine were predominant.

The carbohydrates and amino acids composition of cow bile glycoprotein is detailed.

In 1952, POLONOVSKI and BOURRILLON (19) showed the problems involved in a quantitative study of several components of the bile, because of the complexity and variety of its composition; they summarized valuable literature about the composition of bile and reported new values of bile phospholipids from several species. Other aspects of the bile composition have been studied recently by AZIZ *et al.* (1) (porphyrins); KUENZLE (13) (bilirubin conjugates); ENGLERT (10) (proteins); CLAUSEN *et al.* (6) (acid mucopolysaccharides). With respect to the bile glycoproteins, MAXFIELD *et al.* (16) have studied a periodic acid Schiff-staining macromolecular component of human hepatic bile; and MIAHESCU (17), the seromucoid contents of human bile. DISCHE (7) has found galactose, fucose and hexosamine in human bile; HASHIMOTO *et al.* (12), sialic acid, fucose, galactose and hexosamines in bile mucins from humans, cattle and pigs; BOUCHIER *et al.* (3), sialic acid, fucose, galactose, mannose, glucosamine, galactosamine and the common aminoacids in human bile; and sulfated glycoproteins have been also characterized and isolated from human bile (11, 15). Finally, the nature of the sialic acids from bile of human (5), bovine (4, 5), ovine (5) and porcine (5) species have been determined.

The present paper describes the identification and quantitative determination of oses, hexosamines, and aminoacids (in bovine bile), from the bile of the quoted species.

Materials and Methods

Vesicular bile was employed, except for human specie. 7,500 ml from 21 bladders

of adult cows; 7,100 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 precipitate was obtained by ethanol precipitation (4). After hydrolysis, hexoses were estimated by the procedure of TILLMANS and PHILIPPI (21), and hexosamines by a modification of the ELSON-MORGAN's (9). Aminoacids were determined by the MOORE-STEIN procedure (18), and proteins by the LOWRY method (14). Assays for sulfated were made according to DODGSON (8), and uronic acids by the carbazol procedure (2). Other experimental details have been previously described (20).

Results and Discussion

With some exceptions, the following hexoses and hexosamines were found: D-galactose, D-mannose and L-fucose, D-glucosamine and D-galactosamine, but their concentrations differed according to the species.

The relative concentrations of several aminoacids showed remarkable differences, according to the species. Generally, aspartic + glutamic acids were found in a higher concentration than serine + threonine in the abovementioned species.

After hydrolysis with pronase and filtration through Sephadex or Bio-Gel of several grades, glycopeptides were isolated. Those from the bovine bile were relatively rich in hexoses and contained a lower con-

Table I. *Carbohydrates and aminoacids composition of cow bile glycoproteins.*

	Proportion (% of dry weight)	mmoles per g substance (dry weight)		Relative molar ratio **
Sialic acids (as NANA) *	2.4	0.08		1
Hexoses (Gal+Man)+Fuc	4.4	0.24		3
Hexosamines (Glucosamine+ Galactosamine)	4.4	0.24		3
Glycine		0.79 ***	0.72 ****	10
Aspartic acid		0.71	0.66	9
Glutamic acid		0.71	0.73	9
Alanine		0.58	0.60	7.2
Leucine		0.57	0.54	7
Serine		0.54	0.55	6.7
Valine		0.52	0.45	6.5
Proline		0.49	0.39	6
Threonine		0.46	0.38	6
Arginine		0.39	0.33	5
Isoleucine		0.32	0.28	4
Lysine		0.26	0.23	3.2
Phenylalanine		0.25	0.22	3
Tyrosine		0.21	0.20	3.5
Histidine		0.09	0.05	1
Methionine		0.08	0.07	1
Cyst(e)yne		0.07	0.06	1

* NANA = N-acetylneuraminic acid.

** For amino acids, the relative molar ratios are approximatively calculated according to the values of the hydrolysis after 48 hr.

*** Hydrolysis in 6 NHCl at 105° in a N_2 atmosphere after 48 hr.*** or 72 hr.,**** and correction for losses of threonine and serine (tryptophan is destroyed). Hydrolysis after 24 hr. was incomplete.

centration of hexosamines and very low in fucose.

Table I summarizes the results on the carbohydrates and aminoacids composition of cow bile glycoproteins. Sulfates were also detected, but no uronic acids, in this bile.

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