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Comparative Study on the Composition of Platelets from the Equine, Bovine, Ovine and Porcine Species *

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It is concluded that there are few differences between the concentrations of total protein, total lipids and hexosamines in the blood platelets preparations obtained from the bovine, equine (horse, donkey, mule), ovine and porcine species; for hexoses some differences were noted in two species; with reference to sialic acids the difference varies with the type of the acids: an equal ratio for NGNA and NANA was found in the ovine specie, and NGNA was predominant in the other species (in contrast with the human specie in which only NANA seems to occur). The hydrolysis with *V. cholerae* neuraminidase gave nearly similar results to those obtained by sulfuric acid hydrolysis.

Relatively little information is available regarding the composition of mammalian blood platelets, except for human platelets. Human platelets contain about 50 % proteins, 19 % lipids, the remainder being carbohydrates and salts. Few studies have been made of the carbohydrate fraction, but it is known to contain glycoprotein as well as glycogen. The occurrence of neutral mucopolysaccharides in the granules of platelets was reported in 1953 and was confirmed by MANLEY and MULLINGER (9), in 1967, and WHITE *et al.* (22), in 1970. The ultrastructural localization of acid mucopolysaccharides in human platelets has been studied by SZALONTAY (18), and SPICER *et al.* (16).

The isolation of membranes from human platelets and the determination of their components has been reported by JAMIESON and collaborators (2, 14, 15).

WOODSIDE and KOCHOLATY (23) have determined the concentration of sialic (or acylneuraminic) acids in human and bovine platelets. MADOFF *et al.* (8) have established that the sialic acid of human platelets is the N-acetylneuraminic acid (NANA).*

The present paper describes the iden-

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* NANA = N-acetylneuraminic acid; NGNA = N-glycolilneuraminic acid.

tification and quantitative determination of protein bound sialic acids from the platelets of several mammalian species (except the human specie); in addition, protein bound oses and hexosamines have been determined together with the total lipids and proteins. Our work has been carried out in order to compare the compositions of platelets from different species.

Materials and Methods

Blood from slaughetered animals, as indicated in Table I, was used for the isolation of platelets. The procedure of MULLINGER and MANLEY was employed (12), with little modification: *i.e.* the last button (which consists almost entirely of platelets, > 99.95 %, according to these authors), was washed twice with 0.9 % NaCl and then twice with H_2O.

The hydrolysis for sialic acid was performed with 0.1 N H_2SO_4 at 80° C, for 45 min; the hydrolysis was repeated 4 or 5 times. The 4th and 5th hydrolysis followed homogenization of the platelets, in a tight-fitting all glass Dounce homogenizer. Neuraminidase from *Vibrio cholerae* (Behringwerke) was also used, at 37° for 120 min. For oses, hydrolysis was carried out with 1 N HCl at 100° for 4 hr; and for hexosamines with 3 N HCl at 100° for 4 hr.

Purification of sialic acid from different fractions was generally achieved after filtration through Sephadex G-10; sialic acid was crystallised (in some cases) by acetic acid (6).

The purified sialic acids were identified by descending paper (Schleicher & Schüll 2043 b) chromatography, with the use of the following solvents: a) *n*-butanol-acetic acid-water (4:1:5); b) *n*-butanol-*n*-propanol-0.1 N HCl (1:2:1); c) *n*-butanol-pyridine-0.1 N HCl (5:3:2); and d) ethyl-acetate-acetic acid-water (3:1:3) (v/v). For oses, the solvents c) and a) were the most convenient. Sialic acids were located on paper with p-dimethylaminebenzaldehyde (modified Ehrlich reagent) (3), and oses by $AgNO_3$ solution (20).

For the quantitative determination of sialic acids, the modified resorcinol procedure (11, 17) or the thiobarbituric acid technique (1, 21) was applied; oses were estimated by the procedure of Tillmans and Philippi (19); and hexosamines by a modification of the Elson-Morgan method (5). Proteins were determined by the Lowry method (7); and lipids by gravimetry after exhaustive extraction with chloroform-methanol (v/v, 2:1).

Results

Values of dry platelet residues were between 11.4 %-12.5 % of wet weight depending on the different species (Table I).

The majority of sialic acids were released in the first acid hydrolysis; the second, third and fourth hydrolysis produced progressively lower values, and the fifth gave practically nothing. The hydrolysis with *V. cholerae* neuraminidase gave results nearly similar to those obtained by the sulfuric acid hydrolysis. This suggests a remarkable accesibility of the sialic acids to the action of the neuraminidase, probably because of the extreme position of the sialic acids in the glycoprotein molecules.

Except for the ovine specie, in which NANA and NGNA were detected in approximately the same ratio, NGNA was found as the predominant or unique acyl-neuraminic acid in the other species. (In donkey and mule, a trace of NANA was observed.) Other unidentified neuraminic acid derivatives, probably neuraminoligosaccharides, were detected, principally in horse and calf samples. Total concentrations of sialic acids are summarized in Table I.

Galactose and glucose, in nearly similar amounts, and traces of mannose (except in calf) were found; glucosamine and/or galactosamine were also detected

TABLE I

Platelets components from equine, bovine, ovine and porcine species.

Mean value (4 determinations). The values of the Carbohydrate, Protein and Lipid Fractions are referred as % of dry residue. Between brackets, number of animals from which the sample was pooled.

Specie and age	Platelets		Carbohydrates				Carboh. Fract. (Sialic ac.+Oses +Hexosamines) %	Protein Fract. %	Lipid Fract. %	Carboh.+ Prot.+Lipid %
			Sialic acids		Oses %	Hexosamine %				
	H_2O %	Dry residue %	(*) %	(**) %						
Donkey (10) (young)	87.8 ± 0.60	12.2 ± 0.60	0.96 ± 0.02	0.93 ± 0.03	6.08 ± 0.1	1.04 ± 0.03	8.07 ± 0.15	58.20 ± 0.80	20.40 ± 0.42	86.7 ± 1.37
Horse (6) (adult)	88.5 ± 0.45	11.5 ± 0.45	1.01 ± 0.02	0.96 ± 0.04	6.30 ± 0.1	0.96 ± 0.01	8.24 ± 0.13	64.70 ± 1.05	18.76 ± 0.55	91.7 ± 1.73
Mule (5) (adult)	87.5 ± 0.64	12.5 ± 0.64	0.99 ± 0.04	0.94 ± 0.02	6.50 ± 0.1	1.04 ± 0.02	8.51 ± 0.16	64.08 ± 0.80	21.32 ± 0.43	93.9 ± 1.39
Calf (13)	87.5 ± 0.22	12.5 ± 0.22	1.02 ± 0.03	0.98 ± 0.03	6.67 ± 0.1	1.02 ± 0.02	8.71 ± 0.15	58.65 ± 0.75	22.51 ± 0.48	89.9 ± 1.38
Lamb (20)	88.4 ± 0.25	11.6 ± 0.25	0.86 ± 0.03	0.82 ± 0.04	5.52 ± 0.1	1.41 ± 0.01	7.77 ± 0.14	59.00 ± 0.80	19.25 ± 0.43	86.0 ± 1.37
Hog (12)	88.6 ± 0.30	11.4 ± 0.30	1.00 ± 0.04	0.94 ± 0.02	5.35 ± 0.1	1.04 ± 0.02	7.36 ± 0.16	59.42 ± 0.92	20.10 ± 0.37	86.9 ± 1.45

(*) Sialic acid obtained after sulfuric acid hydrolysis (as NANA).
(**) Sialic acid obtained after neuraminidase from V. cholerae hydrolysis.

in all materials. Their respective concentrations are shown in Table I. No hexuronic acids were detected.

The total lipid and protein percentages are also indicated in Table I.

Discussion

The results above strongly suggest that the predominant sialic acid from the platelets of the species studied here differs from the sialic acid in human platelets [NANA, according to MADOFF *et al.* (8)]. It seems that human materials do not contain NGNA, or else that it exists at a concentration lower than 1 %, compared with other sialic acids; in contrast, other mammalian species contain a high ratio of NGNA, although it varies according to the organ.

The sialic acid concentrations of the platelets assayed in our investigation are very similar. The low values for the ovine specie may be explained by the higher extinction produced by NGNA (predominant in the other species), with the resorcinol method (6). The relative molar ratio of the main carbohydrate components can be calculated as follows: 1 mol of sialic acid: 2 mol of hexosamine (except in the ovine specie, in which it would be 2.5): 10 to 12.5 of hexoses (according to the species). The difference in the concentration of hexoses may be results of the hydrolysis of glycogen.

The incomplete recoveries shown in the last column of Table I would be due largely to mineral salts, principally calcium salts in which platelets seem to be very rich.

It is possible that our values for the components of platelets represent partially lysed platelets, resulting from the last washing steps in the preparative procedure. The carbohydrate concentrations, ranging between 7.3 % and 8.7 %, are close to those (8 %) obtained by NACHMAN and FERRIS (13), and (6 %) by BARBER and JAMIESON (12), for human platelet membranes. The quantity of total lipid that we have found (18.7-21.3 %) agrees with that indicated by MAUPIN (22) of 19 % for human platelets. A comparison between the values of total proteins indicated in Table I (ranging between 58.2 % and 64.7 %) and those for human platelets membranes obtained by other authors [NACHMAN *et al.* (13), 57 %, and BARBER *et al.* (2), 64.5 %] shows that they are similar.

Finally, we have detected a neuraminidase activity in the platelets of the various species (4).

Resumen

Existen algunas publicaciones acerca de la composición química de las plaquetas sanguíneas humanas. Pero escasean los datos sobre la composición de las plaquetas de otras especies de mamíferos. Con una finalidad preferente de Bioquímica Comparada, hemos intentado identificar y/o valorar la concentración de los constituyentes orgánicos principales de las plaquetas obtenidas (en iguales condiciones) a partir de la sangre de las especies siguientes: bovina, equina (caballo, asno, mulo), ovina y porcina.

Se deduce que apenas se encuentran diferencias entre las concentraciones respectivas de proteínas totales, lípidos totales y hexosaminas de las plaquetas procedentes de las especies indicadas. En cambio, sí se hallan diferencias apreciables entre las concentraciones respectivas de hexosas.

Asimismo, se describe por primera vez la existencia de los ácidos siálicos o acilneuramínicos en las plaquetas de las especies equina, ovina y porcina. Las concentraciones de dichos ácidos son próximas entre sí, en los materiales estudiados; por el contrario, existen diferencias considerables en cuanto a su naturaleza. Así, se han encontrado los ácidos N-glicolilneuramínico (NGNA) y N-acetilneuramínico (NANA), en proporciones similares, en la especie ovina; y el ácido N-glicolilneuramínico como predominante mayoritario o único en las restantes especies estudiadas. En todos los casos, dichos ácidos siálicos han sido liberados por hidrólisis mediante neuraminidasa de *V. cholerae* con un rendimiento similar al obtenido mediante hidrólisis ácida.

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