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ISOLATION, CHARACTERIZATION AND CHEMICAL COMPOSITION OF THE MEMBRANE FROM SHEEP PLATELETS

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A procedure of the isolation of platelets from the blood of adult sheep (*Ovis aries* L. var domestica) is reported. This procedure is based on differential centrifugation and a specific lysis for elimination of erythrocytes. We have obtained platelets with a purity at least 99% and a relative high yield (2.3 ± 0.4 g wet/l whole blood ≡235 ± 40 mg platelet proteins/l whole blood). After disruption, homogenisation and ultracentrifugation onto a discontinuous sucrose gradient (1.6 M, 1.1 M, 1.0 M and 0.6 M sucrose), four fractions were obtained. We have separated, for the first time, a particulate preparation enriched in the whole sheep plasma membrane. This fraction was characterized by: (i) the typical membrane morphology as shown by electron micrographs; (ii) the highest activities in membrane marker enzymes such as bis(*p*-nitrophenyl)phosphate phosphodiesterase (EC 3.1.16.1) and 5′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.1.3.35), and the relatively low activity for marker enzymes associated to other subcellular fractions; (iii) the highest sialic acid, cholesterol and phospholipid concentrations. The chemical composition of the platelet membrane isolated is: total proteins, 49%; lipids, 47%; carbohydrates, ≃3.4% (the content of hexoses is twice as high as that of hexosamines and sialic acid). The similarities and differences of this preparation with others from several sources are discussed.

Introduction

Platelet membranes play an important role as receptors and in blood coagulation, hemostasis, aggregation and immunological processes. On the other hand, platelets resemble neurons in some respects and they have recently been proposed as a model for the neuron and for research on psychiatric disorders [1]; their study is of increased interest. However, obtaining platelet membranes is hampered by difficulties in preparing this fraction with enough purity.

Relatively little information is available regarding the isolation and composition of the membranes from blood platelets, except for human platelets. The plasma membrane of human platelets has been isolated [2–4] and its composition, especially for proteins and glycoproteins, has been studied by several authors [3,5–9]; nevertheless, the concentrations of both proteins and lipids reported are, for the same species, significantly different [3,5], probably due to the differences introduced in the isolation procedures of this membrane.

The platelet membrane from pig, another omnivorous species, has also been isolated and characterized [10–12].

We have not found references on the isolation and composition of the membrane from blood platelets of sheep, a herbivorous species. In this paper we describe for the first time: (A) a procedure for the isolation of sheep blood platelets; (B) a technique for the prepara-

Abbreviations PMSF, phenylmethylsulfonyl fluoride; Cbz-Glx-Tyr, *N*-carbobenzoxy-α-L-glutamyl-L-tyrosine; G_{M3}, II^3-NeuAc-LacCer; G_{M1}, II^3NeuAc-GgOse$_4$Cer; NeuAc, *N*-acetylneuraminic acid.

tion of a sheep platelet membrane-enriched fraction; (C) the characterization of this fraction, in comparison with other subcellular fractions from sheep platelets, (i) by electron microscopy, (ii) by marker enzymes, and (iii) by chemical criteria; (D) its chemical composition.

Materials and Methods

Chemicals. *p*-Nitrophenol, the appropriate *p*-nitrophenyl glycosides and phosphate esters, sodium succinate, sodium maleate, Tris(hydroxymethyl)aminomethane, bovine serum albumin, phenylmethylsulfonyl fluoride (PMSF), *N*-carbobenzoxy-α-L-glutamyl-L-tyrosine (Cbz-Glx-Tyr) and *p*-iodonitrotetrazolium violet were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). D-Sucrose was from Panreac (Spain) and all other products were from Probus (Spain). All chemicals were of the highest purity commercially available.

Preparation of platelets. Platelets were isolated from freshly collected blood of adult sheep (*Ovis aries* L. var domestica). The blood was obtained from a local slaughter house and collected into 500-ml polythene vessels containing 75 ml 1.5% disodium EDTA in 0.15 M NaCl as anticoagulant [10]. Within 1 h of collection, the blood was centrifuged in a IEC B-20 centrifuge (rotor A-54) in 250-ml bottles at $300 \times g$ for 15 min at room temperature. The supernatant platelet-rich plasma was separated. It was centrifuged at $370 \times g$ for 6 min when the content of erythrocytes and leucocytes was high; if not, the supernatant was directly centrifuged at $200 \times g$ for 3–5 min, using a swing bucket rotor. The platelet preparation was sedimented at $4300 \times g$ for 15 min and then gently suspended in buffer 1 (10 mM Tris-HCl/0.14 M NaCl/5.5 mM glucose) pH 7.4–7.5, similar to that indicated by Baenzinger and Majerus [4], using 9 ml buffer 1 for the pellet derived from 450 ml blood.

To remove any residual erythrocytes, a specific lysis of red cells was performed at similar conditions to those indicated by Roos and Loos [13]; thus, 1 vol. of the platelet preparation was resuspended into 1 vol. of an ice-cold buffer solution 2 (1.54 M NH_4Cl/0.1 mM disodium EDTA/0.1 M $KHCO_3$) pH 7.4, then 8 vol. distilled water were added and all mixed. After exactly 10 min at 0°C the platelet preparation was diluted with the buffer solution 1 and sedimented at $750 \times g$ for 20 min at 2–4°C. The pellet consists of platelets, and it is microscopically free from other blood cells. Accompanying proteins were eliminated by washing twice the platelets with buffer solution 1 and finally with this solution containing 0.1 mM Cbz-Glx-Tyr and 0.5 mM PMSF [14].

Homogenisation and subcellular fractionation. A platelet whole membrane fraction was prepared by the procedure of Harris and Crawfold [10] as modified by Taylor and Crawford [11], with slight modifications. A ultracentrifuge IEC B-60 was used, at 4°C.

The platelets were resuspended in buffer 3 (5 mM Tris-HCl/0.5 mM PMSF/0.1 mM Cbz-Glx-Tyr) pH 7.4–7.5, containing 0.3 M sucrose, to a total volume of 2 ml for every g of wet platelets. The suspension was homogenised with a Virtis 33 blender operated at top speed for 15-s intervals, followed by 15 s for recooling, in a total time of 5 min. After centrifugation at $4300 \times g$ for 15 min, the unbroken cells were resuspended in the same volume of buffer solution 3 and the homogenisation was repeated at experimental conditions similar to those previously employed. The supernatants of both centrifugations were combined; they are designed as homogenate.

Platelet homogenate (10 ml per tube) was carefully layered onto the upper surface of each sucrose gradient. The following discontinuous layers of sucrose were used: 4 ml of 1 6 M, 11 ml 1.1 M, 6 ml 1.0 M, and 4 ml 0.6 M sucrose. All sucrose solutions were buffered with 5 mM Tris-HCl, pH 7.4–7.5. Centrifugation was performed at $100\,500 \times g_{av}$ for 2.25 h at 4°C in a rotor SB-110, using 40-ml polycarbonate tubes.

The fractions were removed successively from the upper surface by use of a pasteur pipette taking care to avoid contamination by lower levels.

The fractions obtained from the gradient (zonal bands A, B, C and D) were washed with 5 mM Tris-HCl, pH 7.4–7.5, to a sucrose concentration between 0.2 M and 0.3 M; band B was diluted with 5 mM Tris-HCl/0.5 M PMSF/0.1 mM Cbz-Glx-Tyr, pH 7.4–7.5, to a sucrose concentration between 0.2 M and 0.3 M. All fractions were centrifuged in a A-110 rotor at $105\,000 \times g_{av}$ for 1 h at 4°C, yielding particulate (p) and soluble (s) subfractions for each zonal band. The deposited particles were finally resuspended in a

5 mM Tris-HCl buffer (pH 7.4–7.5) for enzyme and chemical analysis.

Electron microscopy. Membrane samples were fixed in 4% (v/v) glutaraldehyde in 50 mM phosphate buffer, pH 7.2, containing 0.2 M sucrose, for 2 h. After washing in 50 mM phosphate buffer, they were postfixed in 1% OsO_4 (dissolved in 50 mM phosphate buffer, pH 7.2), for 2 h at 4°C.

The fixed pellets were washed in this buffer and dehydrated first through graded acetone solutions and later with propylene oxide. Staining was performed with 2% uranyl acetate. The samples were embedded in Araldite, and the sections were stained with lead citrate [15]; finally, they were viewed with a Philips model EM-201 electron microscope.

Assay of enzyme activities. Bis(*p*-nitrophenyl)-phosphate phosphodiesterase (EC 3.1.16.1) was measured essentially as described by Taylor et al. [16]; 0.1 ml of sample was incubated with the substrate, 3 mM bis(*p*-nitrophenyl) phosphate for 30 min at 37°C; 0.2 ml of sample was employed for the assays of both 5′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.1.3.35) and 3′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.1.3.34), and incubations were carried out for 2 h at 37°C.

β-*N*-Acetylglucosaminidase (EC 3.1.3.9) was measured as previously reported [17]. Glucose-6-phosphatase (EC 3.1.3.9) was assayed by the method of De Duve et al. [18] as modified by Jeffrey et al. [19]. Acid phosphatase (EC 3.1.3.2) was determined as described by Taylor and Crawford [11], at pH 5.5. Finally, the method of Pennington [20] was employed to measure succinate dehydrogenase (EC 1.3.99.1).

Chemical analyses. The protein content of the different fractions was determined by the method of Lowry et al. [21] using bovine serum albumin for the standards. The fractions and standards were conveniently diluted to overcome interference by sucrose, and compared with standards prepared with different sucrose concentrations.

Lipids were extracted by the method of Bligh and Dyer as reported by Kates [22]; each lipid fraction was concentrated at 30°C and dissolved in 1 ml of a solvent system containing chloroform/methanol (2 . 1, v/v). The lipid contents were determined by spectrophotometry using Merckotest 3321 (E. Merck). Cholesterol was estimated by the method of Zlatkis et al. as reported by Kates [22]. Phospholipid phosphate was determined after hydrolysis with a mixture of $HClO_4$ and H_2SO_4 [23] by the Bartlett modification of the Fiske-SubbaRow procedure [24]; a factor of 25 was used to convert phosphorus concentration to phospholipid concentration.

Hydrolysis for hexoses, hexosamines and sialic acids were performed with 2 M trifluoracetic acid at 100°C for 5 h, 3 M HCl at 100°C for 5 h and 0.025 M H_2SO_4 at 90°C for 1 h, respectively. Hexoses were determined by the orcinol procedure [25]; hexosamines by the method of Elson and Morgan as described by Davidson [26], and sialic acid by the resorcinol procedure [27] after purification of the samples by chromatography on Dowex 2-X8.

Results and Discussion

Purity and yield of the sheep platelet preparations

The purity of platelets was at least 99%. The elimination of erythrocytes by specific lysis in isotonic NH_4Cl solution seems to contribute to the high quality of the final preparation. Roos and Loos [13] had previously employed this solution in the separation of lymphocytes from peripheral human blood. On the other hand, the disodium EDTA employed as anticoagulant avoids the platelets aggregation and produces better results than other anticoagulants assayed. Nevertheless, EDTA has been described as a solubilizing agent for peripherical proteins; thus, we have not used it in the other steps of the purification procedure.

The method described is very reproducible and permits the isolation of platelets from 6 l of sheep blood in 9–10 h. In addition, the platelets obtained seem to be microscopically intact and to keep their enzymatic activities (see below). The yield (mean value of 17 experiments ± S.D.) was 2.3 ± 0.4 g wet wt/l of whole blood (≡236 ± 40 mg platelet proteins/l of whole blood).

Platelets disruption and sucrose density gradient centrifugation

Because of the small size of the blood platelets and their resistance to mechanical forces, the isolation of the platelet membranes presents particular difficulties. We have assayed several physical methods for platelets disruption such as osmotic lysis with $CaCl_2$,

and glycerol lysis [3]. In our hands, the best results were obtained with the procedure of Harris and Crawford [10], slightly modified, as described in Materials and Methods. About 26% of platelet proteins were found in the homogenate.

After discontinuous gradient centrifugation of sheep platelet homogenate, four fractions were recovered: fraction A, a transparent and slight density fraction, at the 0.3 M sucrose layer; fraction B, a thick and middle density fraction at the 0.6/1.0 M sucrose layers interface; fraction C, a high density fraction, at the 1.0 M/1.1 M sucrose layers interface; and the lower, fraction D, at the 1.1 M/1.6 M sucrose layers interface.

After dilution and centrifugation at $105\,000 \times g_{av}$ for 1 h, two visually distinct regions, soluble phase (s) and particulate phase (p), were obtained. Band B_p was the membrane-enriched fraction (see below). Since the sedimentation and morphological characteristics of fractions A and B_s were very similar, a mixture of both fractions was studied (soluble fraction, named F_s).

The sucrose concentration of the gradient which permits the separation of the sheep platelets membrane (corresponding to the density $d = 1.09–1.12$) is between that ($d = 1.06$) which yields platelets membrane from human blood [3] and that ($d = 1.12–1.15$) which is the most convenient for the isolation of pig platelets membrane [10]. Conditions of platelet disruption, and sucrose concentration of the gradients are two critical points in the preparation of platelets membrane. The fact that several authors have reported different density values as the most convenient for the separation of the human platelet membrane ($d = 1.11$ [28], $d = 1.12–1.13$ [2]) may be the reflection of the peculiar experimental conditions of each isolation procedure.

The isolated membrane fraction that we have obtained corresponds to 10% of the total protein of the homogenate.

Electron microscopic examination

Electron micrographs of the fractions are shown in Fig. 1. The homogenate contains predominantly mitochondria, granules and membranes. The B_p fraction is enriched in membrane, with the typical vesicle appearance. The C_p fraction (a fraction which does not correspond to others obtained from human or pig platelets) contains some mitochondria, granular organelles and membranes; these membranes probably come from the endoplasmic reticulum (see enzymatic characterization). Finally, the D_p fraction consists mainly of granules and mitochondria.

Enzyme activities associated with sheep platelet membrane fractions

Table I shows the distribution of the marker enzymes in the various subcellular fractions: bis(*p*-nitrophenyl)phosphate phosphodiesterase and 3′-dTMP-*p*-nitrophenyl ester phosphodiesterase as external surface membrane markers [11]; 5′-dTMP-*p*-nitrophenyl ester phosphodiesterase as an internal surface membrane marker; β-*N*-acetylglucosaminidase and acid phosphatase for the granular (lysosomes) fraction; glucose-6-phosphatase for the microsomal fraction; and succinate dehydrogenase for mitochondria.

Fraction B_p is enriched 4.3-fold and 2.2-fold in the bis(*p*-nitrophenyl)phosphate phosphodiesterase and 5′-dTMP-*p*-nitrophenyl ester phosphodiesterase activities, respectively, relative to the homogenate. After separation of the external sheep platelet membrane (that we have achieved by centrifugation of the purified preparation of platelet membrane in a continuous sucrose gradient at $405\,000 \times g_{av}$ for 4 h), the activity of the former enzyme was enriched 10-fold.

β-*N*-Acetylglucosaminidase and succinate dehydrogenase activities show very low values in fraction B_p (Table I). By contrast, the acid phosphatase activity of this fraction is higher than in the homogenate. This fact may be interpreted either as the result of the absorption of lysosomal enzymes by membrane structures during homogenisation steps, as it has been previously suggested for pig platelet membrane [11], or as the effect of an association of the putative lysosomal enzymes to the purified membranes [3]. Nevertheless, the validity of some enzymes as markers for platelet subcellular fractions is not well established [10].

Acid phosphatase and β-*N*-acetylglucosaminidase activities are the predominant in fraction C_p, probably due to the high content in granules of this band. Its high glucose-6-phosphatase activity may correspond to the presence of endoplasmic reticulum constituents.

The highest succinate dehydrogenase activity was

TABLE I

SPECIFIC ACTIVITIES OF MARKER ENZYMES IN SUBCELLULAR FRACTIONS FROM SHEEP PLATELETS

Values are the mean of specific activity generally expressed as μmol product released/min per mg protein ± S.D., from six preparations assayed.

Enzyme	Specific activity in fraction						
	Homogenate	A_s	A_p	B_s	B_p (membranes)	C_p	D_p
Bis(*p*-nitrophenyl)phosphate phosphodiesterase (EC 3.1.16.1)	8.82 ± 0.24	2.60 ± 0.10	7.08 ± 0.85	4.25 ± 0.33	37.9 ± 2.6	25.7 ± 1.8	17.4 ± 2.3
5′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.2.3.35)	0.64 ± 0.03	0.66 ± 0.04	0.30 ± 0.01	0.39 ± 0.03	1.43 ± 0.13	1.00 ± 0.12	0.92 ± 0.07
3′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.1.3.34)	0.41 ± 0.02	0.25 ± 0.03	0.36 ± 0.04	0.43 ± 0.06	0.60 ± 0.05	1.16 ± 0.10	1 20 ± 0.19
β-*N*-acetylglucosaminidase (EC 3.2.1.30)	1.10 ± 0.16	1.12 ± 0.22	0.78 ± 0.09	1.36 ± 0.15	0.54 ± 0.06	2.03 ± 0.31	1.40 ± 0.17
Glucose-6-phosphatase (EC 3.1.3.9)	1.00 ± 0.13	0.07 ± 0.07	0.32 ± 0.27	0	1.70 ± 0.40	9.77 ± 1.08	8.66 ± 0.63
Acid phosphatase (EC 3.1.3.2)	26.4 ± 3.7	8.58 ± 0.97	4.21 ± 1.03	5.01 ± 0.75	53.3 ± 5.0	118.0 ± 4.9	124.5 ± 7.6
Succinate dehydrogenase (EC 1.3.99.1) [a]	6.65 ± 1.35	0	0	0	0.35 ± 0.34	11.2 ± 2.4	51.4 ± 5.9

[a] This specific activity is expressed as absorbance at 490 nm/min per mg protein ± S.D.

TABLE II

PERCENTAGE DISTRIBUTION OF ENZYME ACTIVITIES IN THE PLATELETS MEMBRANE FRACTIONS

Values are percentages of homogenate total activity ± S.D., obtained in six assays.

Enzyme	Percentage of enzyme activity in fraction						Recovery (%)
	A_s	A_p	B_s	B_p (membrane)	C_p	D_p	
Bis(*p*-nitrophenyl)phosphate phosphodiesterase (EC 3.1.16.1)	11.7 ± 1.2	2.28 ± 0.55	10.3 ± 1.9	34.7 ± 2.4	10.5 ± 1.7	14.1 ± 2.4	85.7 ± 3.6
5′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.2.3.35)	44.2 ± 4.6	1.30 ± 0.15	11.7 ± 1.3	19.0 ± 1.6	8.47 ± 0.76	8.00 ± 1.68	99.0 ± 2.4
3′-dTMP-*p*-nitrophenyl ester phosphodiesterase (EC 3.1.3.34)	26.4 ± 0.7	2.32 ± 0.36	22.9 ± 2.6	12.5 ± 1.2	9.70 ± 1.07	19.7 ± 3.1	97.0 ± 4.0
β-*N*-acetylglucosaminidase (EC 3.2.1.30)	47.9 ± 4.1	1.54 ± 0.30	23.3 ± 0.9	3.95 ± 0.66	4.33 ± 0.52	5.72 ± 0.94	94.0 ± 3.2
Glucose-6-phosphatase (EC 3.1.3.2)	0	0.71 ± 0.29	0	11.8 ± 2.1	19.0 ± 1.9	64.2 ± 3.2	103.7 ± 3.4
Acid phosphatase (EC 3.1.3.2)	14.7 ± 2.3	0.55 ± 0.22	7.26 ± 1.53	21.6 ± 2.6	21.1 ± 1.2	28.2 ± 5.3	96.7 ± 5.3
Succinate dehydrogenase (EC 1.3.99.1)	0	0	0	0.93 ± 0.67	5.96 ± 1.03	79.4 ± 10.7	87.3 ± 9.7

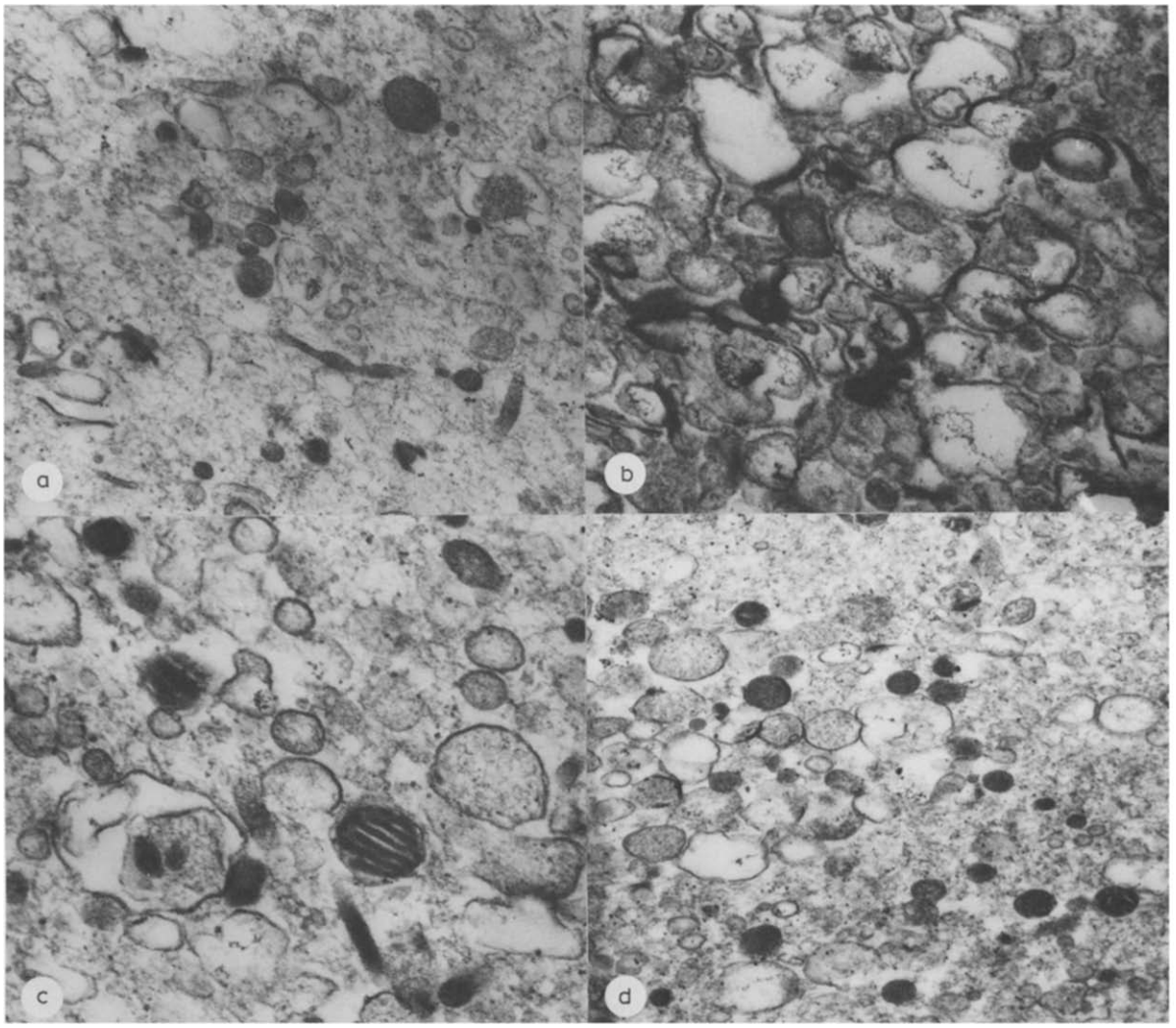

Fig. 1. Electron micrographs of (a) homogenate (magnification × 31 850) showing mitochondria, granules and membranes, (b) membrane fraction (B_p) (magnification × 48 650), (c) fraction C_p (magnification × 48 300) and (d) fraction D_p (magnification × 21 265). Other details are described in the text.

located, as expected, at the fraction D_p (7.7-fold relative to the homogenate) (Table I).

The recovery of all the above mentioned marker enzymes, calculated with respect to the homogenate, was close to 100% (range 86–104%) (Table II).

Characterisation of the platelet membrane by chemical criteria

In addition to morphological and enzymatic criteria, the high concentrations of some membrane constituents such as sialic acid, cholesterol and phospholipids are assumed to be other important data which permit the characterisation of a preparation as an enriched-membrane fraction, at least for membranes from other sources [29].

Table III shows that fraction B_p is the fraction containing the highest values of the above mentioned constituents in comparison with other subcellular fractions.

TABLE III

CHARACTERIZATION OF THE SHEEP PLATELET MEMBRANE BY CHEMICAL CRITERIA

Values are expressed in nmol/mg protein (mean value ± S.D., obtained in four assays). The molecular weight assumed for sialic acid(s) and phospholipids is 314 and 780, respectively.

Fraction	Constituent		
	Sialic acid	Cholesterol	Phospholipid
Homogenate	22.9 ± 2.9	99.5 ± 30.3	75.5 ± 12.7
Soluble ($A + B_s$)	20.4 ± 2.9	26.7 ± 6.9	15.2 ± 5.2
Membrane (B_p)	71 7 ± 6.4	411.5 ± 59.9	365.8 ± 107.4
C_p	54.9 ± 6.8	359.0 ± 15 7	285.0 ± 75.0
D_p	36.5 ± 3.2	261.9 ± 49.2	194.5 ± 42.5

Chemical composition

The content of total protein in the membrane-enriched fraction is about 2 mg/g platelet wet wt. (100 mg platelet proteins).

Table IV shows the results on the chemical composition of several fractions separated from sheep platelets. The recovery is close to 100%.

Details on the yield of chemical components of the fractions obtained from sheep platelet homogenate are indicated in Table V.

It may be deduced that sheep platelet plasma membrane is mainly composed by: proteins, 49%; lipids, 47% (about double concentration of phospholipids than that of cholesterol); and carbohydrates, approx. 3.4% (all expressed as % dry wt.).

In comparison with the platelet membrane from other species, it seems that our sheep platelet membrane preparation contains a protein concentration (49%) similar to that of pig membrane (50%) [11], but its carbohydrate content (3.4%) is lower than that of pig (7–8%) and human (6.9–8%) platelet membranes, its lipid concentration (47%) is higher than that of pig membrane [11].

Finally, we have determined (unpublished results

TABLE IV

CHEMICAL COMPOSITION OF SEVERAL FRACTIONS FROM SHEEP PLATELETS

Mean values ± S.D., obtained in four assays.

Component	Fraction				
	Homogenate	Soluble phase ($A_s + A_p + B_s$)	Membrane	C_p	D_p
Total protein (% dry wt)	77.0	90.2 ± 2.4	48.8 ± 2.6	46.1 ± 13.2	52.3 ± 0.2
Total lipid (% dry wt.)	19.9	7.3	47.4 ± 7.8	46.6	43.4
Total carbohydrate (% dry wt)	2.5	2.3 ± 0.2	3.4 ± 0.3	3.6 ± 0.5	2.4 ± 0.2
Recovery (%)	99.4	99.8	99.6	96.3	98.1
Total phospholipid (μg/mg protein)	58.9 ± 9.9	11.9 ± 4.1	285.3 ± 83.9	222.3 ± 58.7	151.7 ± 32.2
Total cholesterol (μg/mg protein)	38.4 ± 11.7	10.3 ± 2.8	158.8 ± 23.1	138.6 ± 6.1	101 1 ± 19.0
Lipid (μg/mg protein)	220	70	880	900	810
Sialic acid (μg/mg protein)	7.2 ± 0.9	6.3 ± 0.9	22.2 ± 2.0	17.0 ± 2.1	11.3 ± 1.0
Total hexosamine (μg/mg protein)	11.2 ± 2.2	12.4 ± 4.3	21.3 ± 2.6	19.9 ± 4.4	15.0 ± 2.9
Total neutral sugars (μg/mg protein)	14.7 ± 5.4	11.7 ± 3.2	43.6 ± 7.0	69.2 ± 23.4	26.4 ± 2.4

TABLE V

YIELD OF CHEMICAL COMPONENTS OF THE FRACTIONS OBTAINED FROM SHEEP PLATELET HOMOGENATE

Values expressed in percentages (mean value ± S.D., obtained in four assays).

Component	Yield (%) in subcellular fractions				Recovery (%)
	Soluble phase [a] ($A_S + A_p + B_S$)	Membrane (B_p)	C_p	D_p	
Protein	70 1	9.0 ± 0.7	4.6 ± 0.42	5.6 ± 0.9	93.7 ± 4.3
Cholesterol	18.2 ± 4.0	37.4 ± 7.7	18.6 ± 2.9	30.5 ± 6.3	98.3 ± 9.3
Phospholipid	11.6 ± 3.4	37.7 ± 2.6	16.9 ± 2.9	23.9 ± 5.2	92.8 ± 8.7
Neutral sugars	59.5 ± 7.8	18.5 ± 8.5	13.3 ± 3.3	25.2 ± 7.4	–
Hexosamines	56.1 ± 14.5	17.5 ± 4.8	7.0 ± 1.8	14.2 ± 2.0	–
Sialic acid	56.7 ± 5.4	23.0 ± 4.5	10.7 ± 3.6	14.9 ± 3.0	–

[a] The value for A_S fraction is 45.2 ± 3.4, for A_p fraction, 2.53 ± 0.26; and for B_S fraction, 22.4 ± 1.6.

from this laboratory, in collaboration with A. Reglero) the type of gangliosides occurring in the whole sheep platelets. G_{M3} and G_{M1} have been found as the predominant gangliosides. The total gangliosides content of these platelets (expressed in NeuAc) is about 0.12 μg/mg protein.

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