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INDIVIDUAL VARIATIONS OF THE SEVEN CARBOHYDRATE COMPONENTS OF HUMAN ERYTHROCYTE MEMBRANE DURING AGING *IN VIVO*

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ABSTRACT

The contents of fucose, mannose, galactose, glucose, 2-acetamido-2-deoxy-D-glucose and -D-galactose, and sialic acid, when the results were expressed as nmol per mg of membrane dry-weights, were found to be significantly lower in the membranes of old erythrocytes than in the membranes of young ones No significant difference was found between young and old membranes when the compositions were expressed as residues per one hundred carbohydrate residues, suggesting that a homogeneous decrease of the carbohydrate moieties may occur during aging *in vivo*

INTRODUCTION

Various mechanisms have been proposed to describe the early stages of red-cell breakdown, such as cellular fragmentation[1,2] and physical[3] or physicochemical trapping, mediated or not, by a circulating or a membraneous immunoglobulin[4,5] Several changes in the erythrocyte membrane during aging *in vivo* have been studied Among carbohydrate components, sialic acid[6–8] has been the most studied Indeed, in rabbit, rat, man, dog, and duck, neuraminidase-treated, sialic acid-free, or sialic acid-less erythrocytes are removed from the cirulation significantly faster than are intact erythrocytes[9–16] Neuraminidase-treated erythrocytes cannot be used as a model for the study of the molecular events occurring during aging *in vivo*, as the behaviors and the surfaces of neuraminidase-treated and of *in vivo*-aged erythrocytes have been shown to differ[17,18] Furthermore, we previously reported that sialic acid is not the only membrane carbohydrate-component that decreases with aging *in vivo* Total neutral hexoses, fucose, and total hexosamines are also found to be significantly lower in the old human-erythrocyte membrane[7] In the present work, the complete carbohydrate composition of young and old human-erythrocyte membranes was investigated in order to define the overall changes in composition that might occur during aging of human erythrocytes *in vivo*

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EXPERIMENTAL

Separation of erythrocytes of different ages — As the density of erythrocytes is known to increase with aging, human erythrocytes of various ages were separated by centrifugation for 2 h at 2000*g*, on the basis of density differences[7,19] Each sample was treated separately Blood (~100 mL), freshly drawn from healthy donors of A^+ blood-group, was collected in a heparinized blood-bank unit, and then divided into 12–15 glass tubes for centrifugation The buffy coat and the plasma were discarded, and 0 2–0 3 mL of the top and the bottom layers (*i e* , 7–10% of the packed red cells) and 1 mL of the middle layer were removed from each tube by gentle aspiration with a syringe (1 mL) and 18-gauge needle (38-mm) The difference of age between the erythrocytes obtained from the top and those obtained from the bottom layer was assessed by comparing the pyruvate kinase[20] (EC 2 7 1 40) and the cholinesterase (EC 3 1 1 7) activities[21] as previously described[7]

Membrane preparation — Young and old erythrocytes were washed four times with 0 15M sodium chloride The membranes were obtained by hemolysis in hypotonic sodium chloride–phosphate buffer solutions (pH 7 2) according to Hoogeveen et al [22], and they were freeze-dried The weight of sodium chloride–phosphate buffer salts that remained within the ghosts was calculated from the Na^+ and K^+ concentrations of a 2 1 (w/v) membrane suspension in water Na^+ and K^+ were assayed by flame photometry (Instrumentation Laboratory Inc , Lexington, MA 02113, U S A)

Acid hydrolysis of membranes and determination of carbohydrate content — The membranes were submitted to only one hydrolysis by acid for the determination of neutral sugars, hexosamines, and fucose content The conditions used [dried membranes (5 mg) and 2M hydrochloric acid (3 mL) for 3 h at 100° in evacuated sealed tubes] were previously found to be suitable for such studies[23] Neutral sugars and hexosamines were separated and quantitatively determined by ion-exchange chromatography as previously described[24] The total content of sialic acid of dried ghosts (1 mg) was released by hydrolysis with 0 05M sulfuric acid (0 4 mL) for 60 min at 80° The hydrolyzate was made up to 2 mL with water, and deposited on a column of Dowex 1 X-8 (HCO_2^-) anion-exchange resin, and sialic acid was eluted as described by Spiro[25], the eluate was evaporated to dryness in a vacuum evaporator at 40°, the dry residue was suspended in water (1 mL), and sialic acid was determined according to Warren[26]

RESULTS AND DISCUSSION

During aging *in vivo* of human erythrocytes, a significant decrease of each membrane carbohydrate was observed when expressed as nmol of sugar per mg of membrane dry-weight Old-erythrocyte membranes contained, respectively, 86, 87 1, 87 8, 84 8, 87 2, 89 9, and 90 0% of the fucose, mannose, galactose, glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and sialic acid content of young membranes (see Table I) When expressed as percentage of total carbohydrate, the compositions of young- and old-erythrocyte membranes did not significantly differ (see Table II)

TABLE I

CARBOHYDRATE COMPOSITION OF YOUNG-, OLD-, AND MIDDLE-AGED-ERYTHROCYTE MEMBRANES EXPRESSED AS nmol PER mg OF MEMBRANE DRY-WEIGHT

Sugar	*Top layer*[a] *(young cells)*	*Middle layer*[b]	*Bottom layer*[a] *(old cells)*	p *of the coupled differences*			*Ratio of old to young × 100*
				Top and bottom[c]	*Top and middle*[d]	*Bottom and middle*[d]	
Fucose	7 85±0 46	8 6 ±0 28	6 75±0 44	0 001	N s[e]	N s	86
Mannose	10 69±0 52	10 07±0 68	9 32±0 27	0 05	N s	N s	87 1
Galactose	70 95±3 22	64 33±2 07	62 3 ±2 47	0 01	N s	N s	87 8
Glucose	15 48±1 29	12 69±0 86	13 13±1 13	0 01	N s	N s	84 8
N-Acetyl-glucosamine	44 78±1 72	41 78±2 04	39 03±2 17	0 001	N s	N s	87 2
N-Acetyl-galactosamine	29 10±1 22	28 13±1 7	26 17±1 44	0 01	N s	N s	89 9
Sialic acid	33 90±1 31	31 81±2 3	30 54±2 08	0 05	N s	N s	90

[a]These values are mean ±s e m of 13 samples [b]These values are mean ±s e m of 9 samples [c]Comparison of 13 coupled top and bottom values [d]Comparison of 9 coupled middle-and-top, or bottom values [e]N s, not significant

TABLE II

CARBOHYDRATE COMPOSITION OF YOUNG-, OLD-, AND MIDDLE-AGED-ERYTHROCYTE MEMBRANES EXPRESSED AS PERCENTAGE OF TOTAL CARBOHYDRATES

Sugar	*Top layer*[a] *(young cells)*	*Middle layer*[b]	*Bottom layer*[a] *(old cells)*	*Statistical comparison (ε values)*		
				Top and bottom[c]	*Top and middle*[d]	*Bottom and middle*[d]
Fucose	3 71±0 21	3 92±0 15	3 60±0 21	0 045 N s[e]	0 052 N s	0 015 N s
Mannose	5 03±0 20	5 15±0 34	4 99±0 11	0 023 N s	0 070 N s	0 040 N s
Galactose	33 19±0 67	32 92±1 00	33 19±0 63	0 016 N s	0 190 N s	0 160 N s
Glucose	7 23±0 49	6 51±0 46	7 01±0 53	0 064 N s	0 360 N s	0 600 N s
N-Acetyl-glucosamine	21 05±0 52	21 3 ±0 9	20 74±0 78	0 049 N s	0 050 N s	0 070 N s
N-Acetyl-galactosamine	13 69±0 43	14 34±0 78	13 95±0 60	0 058 N s	0 120 N s	0 055 N s
Sialic acid	16 11±0 98	16 31±1 24	16 52±1 28	1 018 N s	0 440 N s	0 370 N s

[a]These values are mean ±s e m of 13 samples [b]These values are mean ±s e m of 9 samples [c]Comparison of 13 coupled top and bottom values [d]Comparison of 9 coupled middle-and-top, or bottom values [e]N s, not significant

Since the demonstration that the sialic acid content of human erythrocytes decreases during aging, the changes of other carbohydrate components have been little investigated The studies to date are either incomplete, as none give the complete carbohydrate-composition, or they report too few samples to be statistically significant The present report describes the complete carbohydrate-compositions of young- and old-erythrocyte membranes, as determined on 13 samples The composition of middle-aged erythrocytes was also established for 9 of these 13 samples Furthermore, as quantitative variations of the carbohydrate content of human-erythrocyte membrane with ABO blood-groups have been described[23], all samples reported here were from the A^+ blood-group

A significant decrease of each membrane carbohydrate-component during aging *in vivo* was observed (Table I) when the compositions are expressed as nmol per mg of membrane dry-weight These results agree with our previous findings, which established that neutral sugars, fucose, total hexosamines, and sialic acid contents of the human-erythrocyte membranes are significantly lower in old erythrocyte than in young ones The decrease reported here for sialic acid (10%), in spite of the different mode of expression of the results, is of the same order of magnitude as that previously established[7,27] The mean composition of the 9 middle-aged-erythrocyte samples is intermediate, between the compositions of young and old, coupled erythrocyte samples

The changes observed for the *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose values, which were lower by 12 8, 10 1, and 12 2%, respectively, in the old red-cell membranes, are of the same order of magnitude as that for sialic acid Greater variations of these three sugar-values have been described by Baxter and Beeley[28], but we used neither the same expression for our results[4], nor the same method for the separation of the erythrocytes

The decrease of mannose (12 9%) and glucose (15 2%) content observed in old, human-erythrocyte membranes is reported here for the first time, as far as we know As mannose residues are known to be present in the core of carbohydrate chains of glycopeptides, the number of such chains in old human-erythrocyte membranes decreases On the other hand, glucose is mainly a component of glycolipids[29] and, to a minor extent, of some glycopeptides[30] Thus, our results suggest that the decrease of membrane carbohydrate components may affect glycopeptides as well as glycolipids

When the compositions were expressed as percentages (Table II), no significant difference was found between young- and old-erythrocyte membranes This result suggests that the decrease of the carbohydrate content of erythrocyte membranes during aging *in vivo* may be homogeneous This suggestion is consistent with the hypothesis of cell fragmentation, and with the elimination of complete carbohydrate-chains rather than with the elimination of individual sugars, as was previously suggested for sialic acid This decrease of the carbohydrate content of the complete human-erythrocyte membrane during aging *in vivo* may be explained, in part, by the decrease ($\sim$10%) of the surface area of old, red blood-cells[3] Various degrees of accessibility

of sialic acid residues[7] and various electrophoretic protein-patterns on polyacrylamide gels have been described[31] for the human erythrocyte aging *in vivo* In addition, such surface-membrane properties as agglutinability, density of antigenic sites, surface-membrane carbohydrates, and auto-rosetting are different for young and old erythrocytes[7 32–35] The observed decrease of carbohydrate components does not disagree with a conformational rearrangement of the membrane and is not incompatible with the modifications of the surface properties during aging *in vivo*

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