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Carbohydrate Constituents of Human Red Cell Stroma.*† (25796)

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Information concerning the carbohydrates in red cell stroma is scanty. Most of the work in this field has been done on specific compounds prepared from stroma. This paper is concerned with analysis of monosaccharides, hexosamines and sialic acid in human red blood cell stroma. In addition, studies were conducted to determine the effect of storage of blood in the cold on carbohydrate constituents.

Methods. Bloods obtained from 4 healthy young men were collected in acid-citrate-dextrose and aliquots were stored in the cold (4°C) under sterile conditions in stoppered bottles. Zero day samples and bloods stored for 15, 30 and 45 days were treated by the short-wash procedure for preparing stroma from dog erythrocytes described by Tishkoff *et al.*(1). This material was lyophilized and kept under vacuum over P_2O_5. Total hexose content was determined by the anthrone method(2). It was found essential to age the anthrone reagent for at least 24 hours to obtain constant results. Dried stroma (10 mg) was hydrolyzed in 3 ml 2N H_2SO_4 in a tube stoppered with a screw-cap lined with rubber. The tube was slowly rotated for one-half hour in a water bath maintained at 96°. Insoluble material was removed by centrifugation and clear supernatant was used for analysis. Galactose in 2N H_2SO_4 was used as a standard and values were expressed as galactose. To separate the lipid-carbohydrate complexes, dried stroma (25 mg) was extracted for one hour with occasional shaking with 10 ml of a chloroform-methanol mixture (2:1)(3) at room temperature. The stroma residue was re-extracted 3 times with one ml portions of the chloroform-methanol mixture; extracts were pooled and evaporated to dryness in a stream of nitrogen. The residual stroma and the extracted material were hydrolysed with 2N H_2SO_4 and analysed for hexoses. To obtain clear hydrolysates, it was necessary to remove the solution carefully so as to avoid the insoluble material which floated on the surface. Hexosamine was determined by the Boas method(4). Stroma (20 mg) was hydrolyzed for 5 hours in 2 ml 2N H_2SO_4 in a screw-cap tube rotated in a water bath at 96°. The hydrolysate was diluted to 8 ml and cen-

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TABLE I. Total Hexose Concentration of Stroma.

	Whole stroma				Lipoidal extract of stroma			
Blood	1	2	3	4	1	2	3	4
Days of storage	g/100 g							
0	2.0	1.8	1.9	1.9	.40 (20)	.43 (24)	.45 (24)	.39 (21)
15	1.9	1.9	1.9	1.8	.41 (22)	.39 (21)	.49 (26)	.43 (24)
30	2.0	1.9	2.1	1.9	.44 (22)	.43 (23)	.47 (22)	.35 (18)
45	1.9	1.7	2.0	1.8	.36 (19)	.36 (21)	.44 (22)	.40 (22)

Figures in parentheses represent % of total hexose.

trifuged. Two ml of this solution was found to be satisfactory for application on the Dowex 50 column. Sialic acid was determined by the thiobarbituric acid assay of Warren(5). Stroma (10 mg) in 2 ml 0.1N H_2SO_4 was heated for one hour in a screw-cap tube while rotating slowly in a water bath at 80°(6). Acetylneuraminic acid, kindly supplied by Dr. Leonard Warren, was used as a standard. The monosaccharides in the stroma hydrolysate were determined by paper chromatography. The first eluate passing through Dowex 50, which is discarded in the Boas method(4), was used. Eluates from 4 runs were combined and concentrated to 5 ml in a desiccator containing NaOH pellets. This concentrated solution was transferred to a 5 ml round bottom beaker and evaporated to dryness in a desiccator; the material was dissolved in 20 μl water and 5 and 10 μl portions applied to Whatman #2 paper. The sugars were chromatographed with an n-butanol-pyridine-water (45:25:40) mixture(7) for 36 hours and developed by spraying with aniline phthalate(8). Reference sugars were run at the same time.

Results. Values for the total hexoses of stroma prepared from freshly drawn blood and from blood stored in the cold for 15, 30 and 45 days are presented in Table I. Hexose concentration is about 2% in 4 different bloods and values are not affected by storage.

TABLE II. Hexosamine Concentration of Stroma Expressed as Glucosamine-HCl.

	Blood			
	1	2	3	4
Days of storage	g/100 g			
0	1.2	1.1	1.2	1.2
15	1.2	1.3	"	"
30	1.3	1.2	"	
45		1.3		1.3

Chloroform-methanol extracts of these stromas contained about 22% of total hexoses (Table I). Lipid-carbohydrate complexes, which are extracted from stroma by fat solvents, have been found to be rich in hexoses (9,10).

Values for hexosamine-HCl concentrations vary between 1.1 and 1.3 g % in the stroma of fresh and stored blood (Table II). It has not been possible to find data in the literature concerning concentration of hexosamine in stroma. McCrae(11) has demonstrated that a mucoprotein isolated from human red cell stroma contains appreciable amounts of hexosamine.

TABLE III. Sialic Acid Concentration of Stroma Expressed as Acetylneuraminic Acid.

	Blood			
	1	2	3	4
Days of storage	g/100 g			
0	1.1	1.2	1.2	1.2
15	"	1.3	"	1.3
30	"	"	"	"
45	1.3	"	1.4	1.2

Table III shows that the sialic acid concentration is about 1% and does not change appreciably during 45 days of storage. The absorption spectrum of the assay chromophore of a number of stroma hydrolysates showed only one maximum at 549 mμ, which indicates that no interfering substances were present. In a few samples sialic acid was determined by the anion exchange resin method of Svennerholm(6) and values were close to those obtained by the Warren method. Klenk and Lempfried(12) demonstrated the presence of about 1% neuraminic acid in stroma of human erythrocytes which is in close agreement with values obtained in the present experiments.

The constancy of concentrations of hexoses, hexosamine and sialic acid of stroma obtained from red cells stored over a 45-day period indicates that these sugars are not involved in the metabolic processes of erythrocytes.

Qualitative analysis of the acid hydrolysates of stroma by paper chromatography showed the presence of large amounts of galactose, smaller amounts of glucose and mannose and a trace of fucose. Excellent resolution without trailing was observed for each of the sugars. A number of investigators(9,10) found galactose and glucose present in stroma. In addition, McCrae(11) found fucose present in his mucoprotein preparation. This appears to be the first study in which mannose is reported to be present in stroma.

Summary. Human red cell stroma has been found to contain 2% hexoses, 1.2% hexosamine and 1.2% sialic acid. These stroma values remain constant during storage of whole blood in the cold for 45 days. Monosaccharides found in stroma were galactose, glucose, mannose and fucose.

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Serumless Medium for Cultivation of Cells of Normal and Malignant Origin.* (25797)

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Development of serum-free media for growth of cells in culture generally has culminated in a medium containing a protein fraction from serum or one suitable for "adapted" cell strains. Waymouth grew strain "L" mouse fibroblasts in media in which Armour fraction V and peptone were substituted for serum(1). Fisher *et al.* successfully grew colonies of human cells in media containing serum albumin and fetuin(2). Lieberman and Ove demonstrated the growth promotive properties of insulin, serum "sticking" factor and catalase in media containing serum albumin(3). Certain lines of strain "L" mouse fibroblasts have been cultivated in completely defined media(4,5,6). Recently, monkey kidney(7), Chang liver cells(8) and mouse lung(9) have been adapted to similar media sometimes supplemented with peptone. The present report concerns growth of a variety of cell cultures in media devoid of serum proteins and without a preliminary period of adaptation.

Methods. One ml of whole medium containing 10,000-15,000 Walker carcinosarcoma 256, KB, human heart, sarcoma 180 cells or 100,000 primary green monkey kidney cells was pipetted into 16 x 125 mm tubes placed in a slanted position in racks. The cultures were established (plated) by incubation for 24 hours at 37°C in an atmosphere of 8%

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