The Carbohydrate Components of Rat Serum Lipoproteins[1] (34395)

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The serum proteins, with the possible exception of albumin, contain covalently linked carbohydrates (1). Scanu (2) and Marshall and Kummerow (3) have analyzed the carbohydrates of high and low density lipoproteins from human serum. Since work on the metabolism of lipoproteins has been carried out mainly with rats, it appeared worthwhile to know the carbohydrate components of serum lipoproteins in this species.

Methods. Serum lipoproteins from rats of the Holtzman strain were isolated by preparative ultracentrifugation and divided into three classes as follows. Serum was centrifuged at 40,000 rpm in a Spinco 50 Ti rotor for 16–18 hours at 10° and the turbid top fraction carefully removed. This fraction was designated as the very low density lipoprotein fraction (VLDL). The infranatant from this centrifugation was then adjusted to density 1.06 with NaBr and centrifuged as before. The top milliliter was removed and designated as the low density lipoprotein fraction (LDL). Finally, the solution was adjusted to a density of 1.21 with NaBr and centrifuged. The top fractions were combined, diluted with an equal volume of NaBr (d 1.21) and recentrifuged. The top milliliter of each tube was removed. This fraction was designated as the high density lipoprotein fraction (HDL). All fractions were dialyzed overnight against 100 vol of 0.15 *M* NaCl. Contamination of the lipoproteins with serum proteins was estimated by measuring the hemoglobin concentration of the original serum and comparing this with that of the lipoprotein fractions. The high absorption of oxyhemoglobin at 412 mμ makes it possible to estimate very small quantities. By this means, it was estimated that the VLDL and LDL fractions contained less than 0.1% of other serum proteins, while the HDL fraction contained 1.2%. This degree of purity was considered acceptable for the purposes of the present work.

Lipids were removed from the dialyzed preparations by treatment with four volumes of ethanol–ether (3:1, v/v), centrifugation, and reextraction two additional times. The precipitate was then resuspended in water, centrifuged, and dried in a vacuum desiccator at room temperature. The precipitates were hydrolyzed for 90 min at 100° in 0.05 *N* HCl, followed by 4 hr at 100° in 1 *N* HCl and 3 hr at 100° in 2 *N* HCl (2). Sialic acid was measured in the 0.05 *N* HCl solution (after precipitation of peptides with 5% TCA) by the method of Warren (4) and identified as *N*-acetyl neuraminic acid by gas–liquid chromatography of the trimethyl silyl ether (5). The combined 1 *N* and 2 *N* HCl hydrolysates were analyzed for neutral hexoses by the phenol–sulfuric acid method of Dubois *et al.* (6) using glucose as a standard. A 4% correction was necessary due to the different color values of galactose and mannose in this reaction. The correction factor was calculated from hexose composition which was determined by gas–liquid chromatography of the alditol acetates (7). The hydrolysates were then evaporated to dryness and analyzed for hexosamine by the Boas method (8). The hexosamine was identified as glucosamine by gas–liquid chromatography of the trimethyl silyl ether (5).

After hydrolysis, the insoluble residue was dissolved in 30% NaOH and analyzed for protein by the micro biuret method (9) using bovine plasma albumin as a standard. The

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TABLE I. Carbohydrate Content of Rat Serum Lipoproteins.

	(mg/100 mg of protein)					
	VLDL		LDL		HDL	
	Prep. 1	Prep. 2	Prep. 1	Prep. 2	Prep. 1	Prep. 2
Total carbohydrate	4.99	4.95	5.29	5.60	2.16	2.23
Neutral hexose	3.50	3.50	3.74	4.08	1.27	1.20
galactose		1.45		1.99		0.50
mannose		1.52		1.71		0.52
glucose		0.53		0.39		0.18
Glucosamine	1.03	1.05	1.12	1.08	1.55	0.60
N-Acetyl neuraminic acid	0.46	0.40	0.43	0.44	0.34	0.43

peptide content of the solutions used for carbohydrate analysis was estimated by the method of Lowry *et al.* (10). All analyses were expressed on the basis of the total protein content of all fractions. In one experiment, the analysis based on the dry weight of the sample agreed within 10% with that obtained by the colorimetric estimation of protein. The small amounts of protein found in the VLDL and LDL fractions made this method of estimation more accurate than that based on dry weight.

Results and Discussion. The results of the analysis of two preparations are given in Table I. They closely resemble those for human serum LDL reported by Marshall and Kummerow (3) who found 3.2% hexose (galactose plus mannose), 1.2% glucosamine, and 0.35% sialic acid. The rat HDL carbohydrate content is similar, but not identical, to that found by Scanu (2) for the human protein. He reported 1.45% neutral sugar (galactose plus mannose in about equal amounts, plus 0.6% fucose), 1.5% hexosamine (not identified), and 0.45% sialic acid. Rat HDL does not contain fucose, does contain glucose, and has about one-third the amount of hexosamine.

As isolated in the present work, the VLDL (which includes chylomicrons) and the LDL appear to have identical carbohydrate components. About twice as much carbohydrate is present in LDL as in HDL (5.2 vs. 2.2%). This does not appear to be the case for human lipoproteins, which all contain about 3.3% of carbohydrate. The significance of the presence of carbohydrate in plasma lipoproteins remains unknown at present.

Summary. The protein of low density rat serum lipoproteins contained 5.2% carbohydrate, consisting of galactose (33%), mannose (31%), glucose (9%), glucosamine (20%), and *N*-acetyl neuraminic acid (8%). The protein of high density rat serum lipoproteins contained 2.2% carbohydrate, consisting of galactose (23%), mannose (24%), glucose (8%), glucosamine (26%), and *N*-acetyl neuraminic acid (18%).

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