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Glycoprotein Content of Serum Lipoproteins.* (25079)

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There is evidence to suggest a relationship between glycoprotein content of arterial wall on the one hand and aging process and arterial disease on the other. Thus, the arterial intima and media stain increasingly for mucopolysaccharides with age and in presence of atherosclerosis(1,2), even though chemical determinations have not shown an increase in hexosamine concentration of arterial tissue with age or arterial disease(3-5). Another possible link between glycoproteins and arterial disease is provided by relationship between lipemia clearing factor, probably a heparin-containing protein(6), and lipid metabolism. An inhibitor of lipemia clearing factor containing hexosamine has also been demonstrated(7). Hexosamine and glycoprotein content of serum itself is said to increase with age and in presence of atherosclerosis(8). Finally, glycoproteins of ground substance appear to be in equilibrium with the serum(9). Since not only glycoproteins, as discussed, but also lipids play a part in processes associated with atherosclerosis and do, indeed, coexist in the lesion itself, it seemed of interest to study their interrelationship in serum from which the arterial deposits may be in part derived. Present studies were undertaken to determine glycoprotein content, expressed as hexosamine concentration, in lipoproteins of human serum. If glycoproteins and lipoproteins were linked in the serum, these substances might be simultaneously deposited in the arterial wall after infiltration. The current studies suggest that serum glycoproteins and lipoproteins are not linked to any appreciable extent. It would seem likely, therefore, that glycoproteins in the arterial wall are either deposited from serum independent of lipids, or are derived wholly or in part from local alterations in the arterial wall.

Methods. Blood was obtained from 4 healthy blood donors A, B, C, and D. Blood was allowed to clot in dry flasks and serum separated. All operations were carried out in the cold as far as possible during all stages. Lipoproteins were separated in

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TABLE I. Hexosamine Concentrations in Lipoprotein Fractions of Human Serum.

	Hexosamine concentration in mg/100 ml of native serum.			
Donors	A	B	C	D
Total serum	78	89	84	86
I. Chylomicrons	.84	3.04	1.68*	.05†
Total lipoproteins (α & β)			1.89	1.68
Infranates	76.5		102.2	73.5
II. Chylomicrons			5.1 *	.05†
β Lipoproteins		3.08	3.1	2.0
α "		.85	.24	.21
Infranates			69.2	77.3

* Not respun. † Identical experiment.

Spinco Model L ultracentrifuge using rotors 30 or 30.2 at approximately 79,000 x gravity or rotor 40 at 105,400 x gravity. In runs A and B, chylomicrons were not spun off separately. In runs C and D, chylomicrons were removed first after layering serum with normal sodium chloride, spinning in rotors 40 and 30.2, respectively, for 120 minutes and removing top fraction with tube slicer. Native serum in runs A and B and chylomicron infranatants in runs C and D were then adjusted to densities of 1.21 and 1.063, respectively, using the formula of Havel, Eder and Bragdon(10) for calculating requisite amounts of NaCl and KCl. The solutions were then spun in rotor 30 for 14 hours and the top fractions again separated in tube slicer, removing all lipoproteins where density of 1.21 was employed and β lipoproteins (with or without chylomicrons) where tubes were spun at density of 1.063. In the latter case, more salt was added to bring the density to 1.21 for further separation of α lipoproteins; these were again spun 14 hours in rotor 30 and top fraction removed in tube slicer. All top fractions containing lipoproteins were recentrifuged 14 hours with salt solution of requisite density using rotor 40 or 30.2, to remove lipoprotein contaminants. This was usually achieved after 2 respinnings. Respinning was carried out in each instance except in chylomicrons in run C. All infranatants in runs A, C and D were carefully collected and measured. Hexosamine was determined by Rimington's modification of Elson-Morgan procedure(11) in all lipoprotein fractions and infranatants, as well as native serum. From knowledge of hexosamine concentrations and total volumes, amounts of hexosamine could be determined in each specimen. In reporting results, all figures were converted to amount of hexosamine/100 ml of native serum. In calculating total hexosamine infranate concentrations, hexosamine in the lipoprotein respin infranates was included among the non-lipoprotein hexosamine. All final infranatant solutions were free of cholesterol as determied by the method of Abell and associates (12).

Results. The data indicate that concentration of hexosamine in lipoprotein fractions is small (Table I). The proportion of total serum hexosamine contained in lipoprotein fractions was consistently less than 5%, except in second part of run C, in which the chylomicrons had not been respun and were, no doubt, admixed with serum.

Hexosamine recoveries were calculated for 5 runs in which infranates were collected; their range was between 83 and 126% with mean of 98%. These recoveries appear not unreasonable in view of the inherent errors involved in repeated volumetric measurements and in the chemical method itself. It is concluded that no appreciable amounts of hexosamine were lost from the lipoprotein fractions during their physical separation since, in slicing the tubes, the cut was always made slightly below the line of demarkation between supra- and infranatants.

Comments. The present data differ from those recently reported by Kirby(13) who found that a major proportion of human serum mucopolysaccharides were precipitated in association with lipoproteins in Cohn frac-

tions III and IV-1. The discrepancy between her results and ours may be due to the difference in methods used. Kirby's results suggested but did not prove that there might be a linkage between lipo- and glycoproteins. Our data do not suggest existence of major linkages of this kind and seem to indicate that the glycoproteins in the arterial wall are either deposited from serum independently rather than alongside the lipoproteins or arise from local synthesis.

Oncley also believes that lipoproteins are essentially carbohydrate-free, but stresses lack of specific methods for detection of these substances in this situation(14). It is possible that even the small amount of lipoprotein-hexosamine detected in our experiments does not reflect the presence of mucopolysaccharides. Findings similar to present data have also been reported by Heide and coworkers(15).

Our results should not be taken to indicate that there is no association whatever between lipoproteins and glycoproteins in serum or tissue. While amount of glycoprotein, expressed as hexosamine associated with lipoprotein fractions was relatively small, glyco-lipid complexes may still be of importance in certain phenomena relating to lipid transport and deposition.

Summary. The amount of glycoprotein, expressed in terms of hexosamine concentration, was determined in lipoprotein fractions of 4 human sera, separated in ultracentrifuge at various densities. On an average, no more than approximately 5% of total serum hexosamine was detected in lipoprotein fractions.

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Some Compounds Active Against Experimental Visceral Leishmaniasis.* (25080)

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In exploring further usefulness of a new 8-day method for screening compounds for activity against visceral leishmaniasis(1) we have shown that cotton rats, chinchillas, and even mice can be used equally as well as golden hamsters(2). Preliminary experiments have also shown a marked activity of amphotericin B, and its more soluble form Fungizone, against this infection in hamsters (2). That polyenes might be effective against leishmanial infections was also suggested by Grossowicz and Rasooly(3) on the basis of their powerful trypanostatic effect *in vitro* against *Herpetomonas culici-*

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