The interaction of plasma glycosaminoglycans with plasma lipoproteins

Yasuhide Nakashima, Nicola Di Ferrante, Richard L. Jackson, and Henry J. Pownall

From the Laboratories of Connective Tissue Research of the Department of Biochemistry; from the Departments of Medicine and of Surgery, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77025

SUMMARY

Modifications of existing methods have allowed for the isolation and purification of various species of plasma glycosaminoglycans on the basis of their sulfate content and molecular size. All of the preparations precipitated human plasma low density lipoproteins (LDL); maximal precipitation occurred with amounts of glycans corresponding to 50 μg of hexurionate and 12 μg of LDL.

The interaction of glycans with pyrene-labeled lipoproteins was also studied, measuring variations of the fluorescence emitted by the monomer (M) and excimer (E) species of the bound pyrene. The ratio I/IM is proportional to c/η, where c is the microscopic concentration of the pyrene confined to the hydrocarbon region of the lipoprotein and η is the micro-viscosity of that region. To 0.12 mg of pyrene-labeled LDL, very low density lipoproteins (VLDL) or high density lipoproteins (HDL) were added increasing amounts of the various glycan preparations. The sulfate-rich species decreased the I/IM ratio of LDL and HDL but not that of VLDL. This finding suggests that the glycan caused a change in lipoprotein conformation associated with either an increased volume or increased microscopic viscosity of the hydrocarbon region. The modification of LDL conformation could be prevented by proteolytic treatment of the sulfate-rich species or by addition to the system of suitable amounts of sulfate-poor species or of chondroitin-4-sulfate, but could not be prevented by increased ionic concentration. These results suggest that the two main species of plasma glycans are important in maintaining adequate rheological properties of plasma lipoproteins.

The interaction between plasma lipoproteins and macromolecular polyanions has been studied by many authors with a variety of ingenious techniques (1-10). The presence of glycosaminoglycans in various cellular elements and extracellular spaces of the arterial wall, their postulated role in the structure and function of the vascular walls, their physicochemical properties, and their presence in blood (11-16) have prompted detailed studies of their interaction with lipoproteins. In view of the determinant role ascribed to lipoproteins, and in particular, to the low density lipoproteins, in the pathogenesis of early atherosclerotic lesions (17-19) the relevance of their interactions with glycosaminoglycans has been the object of protracted speculations (9, 10, 20, 21).

In the present report, we have studied the interaction of plasma glycosaminoglycans with very low density lipoproteins, low density lipoproteins, and high density lipoproteins. The low levels of circulating glycosaminoglycans dictated that meaningful experiments be carried out with minimal amounts of them and with physiologically corresponding quantities of lipoproteins.

Our previous studies (22) have demonstrated that plasma glycosaminoglycans consist essentially of chondroitin-4-sulfate, which may be separated into two different types on the basis of ester sulfate content: those capable of binding as such to ECTEOLA* anion exchange-nitrocellulose (operationally defined as "free" glycans, and having an average molar ratio 0.68, sulfate to hexosamine), and those able to do so only after treatment with proteolytic enzymes (defined as "bound" glycans, and having an average molar ratio of 0.48, sulfate to hexosamine).

For this study, it was necessary: (a) to harvest sufficient amounts of both types; (b) to isolate bound glycans without the use of proteolytic enzymes; and (c) to use methods capable of detecting their interaction with lipoproteins at levels lower than those forming precipitates.

EXPERIMENTAL PROCEDURE

Materials

Plasma was obtained from the Blood Bank of the M. D. Anderson Hospital and Tumor Institute. ECTEOLA-cellulose (0.25 meq/g) was from Carl Schleicher and Schuell Co. and UM-2 Diaflo membranes from Amicon Corp. Crystalline papain was from Nutritional Biochemicals Corp.; pyrene from Aldrich Chemical Co.; SP sulfopropyl-Sephadex C-50, Sepharose 4B, and DEAE-Sephadex A-50 from Pharmacia; Bio-Gel P-6 (50 to 100 mesh), Bio-Gel A 50m (50 to 100 mesh), and Bio-Glass 500 (glass beads) from Bio-Rad. Heparan sulfate (Preparation R01-2323/754) was a gift from Dr. K. von Berlepsch, Hoffmann-La Roche, Basle.

1 The abbreviations used are: ECTEOLA, epichlorohydrin triethanolamine; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; PPL, light fractions of proteoglycans; I, monomer fluorescence of pyrene bound to lipoproteins; IE, excimer fluorescence of pyrene bound to lipoproteins.
Switzerland and dermatan sulfate from Dr. J. A. Cifonelli, University of Chicago. Other standard glycosaminoglycans were from commercial sources: chondroitin-4-sulfate from Nutritional Biochemicals Corp., chondroitin-6-sulfate from Miles, hyaluronic acid from Worthington, and sodium heparinate from Cibbiochem. All reagents, reagents grade, were obtained from commercial sources.

**Methods**

**Isolation and Purification of Plasma Glycosaminoglycans**

These were performed at room temperature as follows.

**Free Glycans**—Human plasma (100 ml) was passed through a column (2 X 25 cm) of ECTEOLA-cellulose equilibrated with 0.15 M NaCl. Thereafter, the column was washed with 300 ml of 0.15 M NaCl; the effluent and wash were combined, and used to prepare the bound glycans (see Scheme 1). After an additional wash with 1200 ml of 0.15 M NaCl, the ECTEOLA column was eluted with 4.0 M NaCl. The eluates, containing hexurionate, were dialyzed against distilled water and concentrated by ultrafiltration with a UM 2 Diaflow membrane. The free glycans were then purified on a column (2 X 14 cm) of SP-Sephadex C-50 medium, as described by Berman (23). The hexurionate-containing effluent, dialyzed and concentrated by ultrafiltration, was applied to a column (0.9 X 90 cm) of Bio-Gel P-6 packed with distilled water. Bound Glycans—One-fourth of the effluent from the ECTEOLA column (1000 ml of run-through plasma + 300 ml of 0.15 M NaCl wash) was passed through a column (4.5 X 40 cm) of DEAE-Sephadex A-50 equilibrated with 0.15 M NaCl, pH 5.0. It was then eluted with 2.0 M NaCl; the fractions containing hexurionate were dialyzed, concentrated, and desalted by ultrafiltration, and further purified on SP-Sephadex C-50 and on Bio-Gel P-6.

**Preparation of Proteoglycans from Bovine Nasal Septa**

This was performed at 4° according to the "dissociative" method of Sajdera and Hascall (24). The product was fractionated by ultra centrifugation as described by Gerber et al. (25), and the light fraction (PPL) was used. Its uronic acid content was 27.9%, dry weight.

Analytical data of the various glycosaminoglycans used have been published previously (26).

**Papain Digestion of Plasma Glycans**

Aliquots of the various plasma glycans (200 μg of hexuronic acid each) were dissolved in 5 ml of 0.1 M potassium phosphate buffer, pH 6.4, containing 7 mg of EDTA, 4 mg of cysteine-HCl and 100 μg of crystalline papain. After incubation at 50° for 18 hours, the digests were deproteinized on SP-Sephadex C-50 (23), and the hexuronate-containing effluents were desalted and lyophilized.

**Molecular Weight Determinations**

The molecular weights of the free and bound glycans retained by Bio-Gel P-6 were determined by gel filtration on a column (0.9 X 90 cm) of Bio-Gel A-50m equilibrated with 0.025 M NaCl, according to the method of Constantinoupolou et al. (27); the column was calibrated with standard glycans of known molecular weight (28). The molecular weights of the free glycans excluded from Bio-Gel P-6 were measured, before and after papain treatment, by sedimentation equilibrium (29) at 10° and 20°, utilizing speeds of 17,250, 21,740, and 28,000 rpm, and an assumed value for the partial specific volume of 0.60 ml/g (30, 31).

**Preparation of Lipoproteins**

VLDL was prepared by plasmapheresis from plasma of patients with hyperlipoproteinemia type IV (32). LDL and HDL were prepared from plasma of fasting, normal donors by ultracentrifugal flotation, using KBr solutions of the following density limits: 1.020 to 1.050 for LDL, and 1.063 to 1.210 for HDL (32). Each lipoprotein preparation, washed twice in KBr solutions of appropriate density, was dialyzed at 4° against a "standard buffer," consisting of 0.140 M NaCl and 0.0027 M CaCl₂, adjusted to pH 7.4 with 0.5 M NaHPO₄. The criterion of purity of each lipoprotein was determined by immunodiffusion techniques using specific antisera. VLDL formed precipitin lines with anti VLDL and LDL immunserum, but not with anti-HDL immunserum; LDL formed precipitin lines with anti-LDL immunserum, but not with anti-HDL immunserum; HDL formed precipitin lines only with its corresponding antisera.

**Labeling of Lipoproteins with Pyrene**

Immediately before use, lipoproteins were labeled with pyrene at room temperature with one of the following methods. In the first (33), which is useful for labeling LDL but not VLDL or HDL, 5 g of Bio-Glass 500 beads were stirred into 50 ml of chloroform containing 4 mg of pyrene. The solvent was removed by flash evaporation at room temperature, and 0.5 g of the dry beads was used to prepare a column to which was applied 5 ml of LDL solution containing 1 mg of cholesterol/ml (corresponding approximately to 2 mg of LDL/ml). The LDL-containing effluent was diluted with standard buffer to give a final cholesterol concentration of 0.12 mg/ml; this solution contained pyrene equal to 4% of the LDL by weight.

In the second method, which is suitable for labeling VLDL, LDL, or HDL, 2 mg of pyrene in 1 ml of absolute ethanol were added to 49 ml of the standard buffer and mixed vigorously. One milliliter of this suspension, mixed with 1 ml of lipoprotein (1 mg/ml), was immediately applied to a column (0.5 X 1.0 cm) of Sepharose 4B and eluted with the standard buffer; the eluent contained 0.5 mg of lipoprotein/ml, with a pyrene concentration equal to 4% by weight.

**Interaction between Lipoprotein and Glycosaminoglycans**

Aliquots of plasma glycan solutions or of a heparin solution, containing from 30 to 100 μg of hexurionate, were lyophilized. To each sample were added 4 ml of unlabeled LDL (equal to 6 mg of cholesterol) in standard buffer, and the tubes were left at 23° for 30 min. The precipitate formed was pelleted by centrifugation at 4°, for 20 min at 900 X g, washed once with 5 ml of standard buffer, and collected again by centrifugation. Thereafter, it was dissolved in 0.5 ml of 10% NaCl and used for cholesterol determination.

In other experiments, to 0.12 mg of pyrene-labeled lipoprotein in 0.5 ml of standard buffer were added increasing amounts of plasma or standard glycans in 0.10 ml of H₂O (from 0.05 μg to 2.5 μg, in terms of hexurionate). After 3 hours at 37°, fluorescence was measured against a blank containing the same amount of pyrene-labeled lipoprotein and 0.10 ml of H₂O. Fluorescence was measured against an Amino-Bowman spectrophotometer (excitation at 320 nm) with a 5-mm slit; emission was recorded with a 4-mm slit from 360 to 550 nm. The microcuvettes (3 X 3 X 20 mm) were maintained at 37° during the readings.
Other Procedure

Hexuronate was measured by the m-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (36), protein by the method of Lowry et al. (37), and cholesterol with the Lieberman-Burchard reaction (38). In the present experiments, the amounts of lipoproteins used were calculated as follows. VLDL was estimated on the basis of 10% protein content, and LDL and HDL on the basis of 50% and 33% cholesterol content, respectively (19).

RESULTS

Isolation and Purification of Plasma Glycosaminoglycans—Free and bound plasma glycans were isolated and purified as outlined in Scheme 1. The free glycans were eluted from Bio-Gel P-6 (Fig. 1) in two broad peaks: Fraction I, emerging at the void volume (high molecular weight free), and Fraction II, less sharply defined, being retained (low molecular weight free). Rechromatography of the latter on the same column (Fig. 1, inset) yielded an asymmetrical peak, suggesting various molecular species. Both peaks were utilized in our experiments. The bound glycans were eluted from Bio-Gel P-6 (Fig. 2) in two peaks, a very small one, which eluted at the void volume and was discarded, and a major one, which was retained in the gel, and was subsequently utilized.

Table I lists the average molecular weights and concentrations of the isolated glycans. Detailed analytical data of purified free and bound glycans have been published previously (22).

Interaction between Lipoproteins and Glycosaminoglycans—Table II shows that preparations of plasma glycans gave optimal precipitation of LDL at a concentration of 50 µg (as hexuronate); however, none of them was as effective as sodium heparinate.

Using pyrene-labeled lipoproteins, two fluorescence maxima were observed: the monomer at 390 nm, and the excimer at 470 nm. Fig. 3A shows the fluorescence of increasing amounts of 4% pyrene-labeled LDL; Fig. 3, B and C shows the spectra of 0.12 mg of VLDL and HDL labeled with 2% and 4% pyrene, respectively.

The ratio of \(I_E/I_M\) fluorescence of 4% pyrene-labeled LDL incubated with standard preparations of glycosaminoglycans with PPL and with free and bound glycans is shown in Fig. 4. While the standard preparations, including heparin and PPL, did not significantly change the \(I_E/I_M\) ratio of LDL, a substantial decrease was observed (Fig. 4B) when LDL was incubated with high or low molecular weight free glycans (maximum change, 61% of the original value). Under the same conditions, corresponding amounts of bound glycans were without effect.

Besides LDL, also HDL and VLDL were incubated with increasing amounts of high or low molecular weight free and bound glycans (Fig. 5). While LDL and HDL show obvious spectral changes when incubated with either high or low molecular weight free glycans, they remain unchanged upon incubation with bound glycans. Pyrene-labeled VLDL failed to demonstrate any appreciable spectral change when incubated with either free or bound glycans. Fig. 6 shows that the effect of high molecular

| Table I | Molecular weight and concentration of plasma glycans |
|---------|-----------------------------------------------------|
| Plasma glycans | Molecular weight | Micromolars of hexuronate/10 ml of plasma | Protein/hexuronate, w/w |
| Free | High molecular weight | 37,100 ± 2,000 | 3.39b (2.80 ~ 4.13) | 0.93 |
| After papain digestion | 18,500 ± 1,500c | 2.97b (2.00 ~ 4.18) | 0.74 |
| Low molecular weight | 5,320c | 0.00 | 2.2 |
| Bound | 2,800c | 34.9b (21.94 ~ 42.55) | 2.2 |

a Determined by sedimentation equilibrium. Small amounts of material of approximately 30,000 molecular weight were also present before papain treatment.
b Average value of repeated analyses and their range.
c Determined by gel filtration with Bio-Gel A-50m calibrated with standard glycosaminoglycans.

| Table II | Precipitation of LDL (corresponding to 6 mg of cholesterol) by various plasma glycans and sodium heparinate |
|---------|-------------------------------------------------------------|
| Glycosaminoglycans | Amount of glycans in reaction mixture (50 µg of hexuronate) | Amount of sodium heparinate (50 mg) |
| | 30 | 50 | 100 |
| Free glycans | | | |
| High molecular weight | 6.5 | 158 | 35 |
| Low molecular weight | 18.0 | 62 | 0 |
| Bound glycans | 19.0 | 47 | 0 |
| Sodium heparinate | ND | 902 | 129 |

a N.D., not performed.
Free and bound plasma glycosaminoglycans, prepared without the use of proteolytic enzymes, were obtained in yields comparable to those of the previous method (22). Gel filtration and ultracentrifugal analyses of these preparations have demonstrated considerable heterogeneity. The high molecular weight free glycans probably consist of two chains attached to a peptide backbone, since treatment with papain reduces their average molecular weight from 37,100 to 18,000.

While the amount of high molecular weight bound glycans was insufficient for study, the low molecular weight fractions of both species were utilized in the present report. Their molecular parameters suggest that they represent oligosaccharides still attached to protein.

The availability of sufficient amounts of plasma glycans has permitted a study of their interaction with the various lipoproteins and particularly with LDL, the species which has been demonstrated to interact preferentially with various polyanions (1) and with the arterial wall (8).

The experiments have been performed with two levels of reactants. In the first series (which is comparable to experiments performed in the past in various laboratories), 12 mg of LDL were combined with 30 to 100 μg of glycans, as hexuronate, in order to verify the occurrence of interaction between the two species of macromolecules. The results presented in Table II indicated...
that all of the species tested are capable of precipitating LDL, 50 \( \mu g \) of each type being the most effective amount.

In the second series of experiments, the use of pyrene-labeled lipoproteins allowed us to use minimal amounts of reactants and to approach their physiological proportions. The parameter measured, the ratio of intensities \( I_E/I_M \), is directly proportional to \( \eta_i \), the viscosity of the hydrocarbon region of the lipoprotein (39, 40). Thus, a decrease in the exomer to monomer ratio observed in pyrene-labeled lipoproteins must result from a change in the microscopic structure of the lipoprotein, although it is not possible at this time to distinguish the relative importance of changes in microviscosity or hydrocarbon volume in producing this effect. If one visualizes the pyrene in

**Fig. 6.** Effects of increasing ionic strength on the interaction between 0.12 mg of 4% pyrene-labeled LDL and free glycans. A, value of \( I_E/I_M \) ratio for LDL control; B, its decrease upon incubation with 1.5 \( \mu g \) of high molecular weight free glycans under standard ionic conditions (0.14 M NaCl); C, its decrease when, after such incubation, the NaCl concentration was raised to 0.9 M and incubation was resumed for 3 hours; D, its decrease when incubation was carried out directly in 0.9 M NaCl. Control samples were identical in composition to experimental ones, glycans being omitted.

**Fig. 7.** Effects of proteolytic treatment of free and bound glycans on their ability to change \( I_E/I_M \) ratio of 0.12 mg of 4% pyrene-labeled LDL. High molecular weight free glycans, before (○---○) and after (●---●) papain treatment; low molecular weight free glycans, before (△---△) and after (△---△) papain treatment; bound glycans, before (■---■) and after (■---■) papain treatment.

**Fig. 8.** Inhibitory activity of bound glycans and of chondroitin 4-sulfate on effect of free glycans on \( I_E/I_M \) ratio of 0.12 mg of 4% pyrene-labeled LDL. Different amounts of indicated preparations were incubated with 0.8 \( \mu g \) of high molecular weight free glycans (A) or low molecular weight free glycans (B), and 0.12 mg of pyrene-labeled LDL for 3 hours. Values of \( I_E/I_M \) ratio were compared with those of LDL standard and of a sample containing LDL + free glycans.
the hydrocarbon region of the phospholipids and cholesterol ester layers of lipoproteins, an increased viscosity or swelling of this region (which would decrease the microscopic concentration of pyrene, c) could reduce the rate of eximer formation by decreasing the monomers' collision rates (39, 40).

The results shown in Figs. 4 and 5 indicate that the ratio $I_E/I_M$ of solutions containing either LDL or HDL decreases considerably upon interaction with high molecular weight and low molecular weight free glycans at a physiological pH and ionic concentration and with quantities of reactants approaching their physiological levels (250 to 500 μg of glycans as hexuronate, and 200 mg of LDL/100 ml of plasma). Similar changes in the $I_E/I_M$ ratio have been obtained with LDL labeled with 2% pyrene. The fact that only a very limited decrease of the $I_E/I_M$ ratio is observed as a result of interaction of LDL with large amounts of heparin (which is recognized to be the most efficient precipitant for LDL (8)) suggests that the formation of soluble and insoluble complexes between lipoproteins and glycans may not involve the same mechanisms. In fact, contrary to the formation of insoluble complexes (9, 10), the interaction between free glycans and LDL is not eliminated by a 6-fold increase in ionic concentration (Fig. 6), suggesting that it is not simply an electrostatic interaction. The drastic effects elicited with protolytic treatment of both high molecular weight and low molecular weight free glycans (Fig. 7) are consistent with this assumption, and suggest that, under the conditions of our experiments, protein-protein interactions might have occurred. Because of the low protein content of VLDL, its lack of interaction with the LDL particles and contribute to their permanence within the arterial wall (40).

Finally, the favorable results claimed by Morrison (41) and by Nakazawa et al. (42) with the oral and parenteral administration of chondroitin-4-sulfate to experimental animals and patients with atherosclerosis might find a rational explanation in the protective effects that this glycan and its products of extensive degradation, such as the bound glycans, show in preventing the molecular modifications of LDL caused by plasma-free glycans.

Acknowledgments—We acknowledge the valuable assistance and advice of Drs. Antonio M. Goto and Louis C. Smith, and the repeated gifts of human plasma from the Blood Bank of the M. D. Anderson Hospital and Tumor Institute of the University of Texas. The ultracentrifugal analyses were kindly performed by Dr. Kirk C. Aune.

REFERENCES

1. Bernfeld, P., Donahue, V. M., and Beerkowitz, M. E. (1957) J. Biol. Chem. 226, 51-64
2. Aments, J. S., and Waters, L. L. (1960) Yale J. Biol. Med. 33, 112-121
3. Gero, S., Gergely, J., Devényi, T., Jakab, L., Székely, J., and Virag, S. (1960) Nature 187, 152-153
4. Gero, S., Gergely, J., Devényi, T., Jakab, L., Székely, J., and Virag, S. (1961) J. Atheroscler. Res. 1, 67 71
5. Bihari-Varga, M., Gergely, J., and Gero, S. (1964) J. Atheroscler. Res. 4, 106-109
6. Bihari-Varga, M., and Végh, M. (1967) Biochin. Biophys. Acta 144, 202-210
7. Tracy, R. E., Dzioga, K. R., and Wissler, R. W. (1965) Proc. Soc. Exp. Biol. Med. 118, 1085-1098
8. Shinivasan, S. R., Lopez-S, A., Radhakrishnamurthy, B., and Berenson, G. S. (1970) Atherosclerosis 12, 521-534
9. Shinivasan, S. R., Dolan, P., Radhakrishnamurthy, B., and Berenson, G. S. (1972) Atherosclerosis 16, 95-104
10. Iverius, P. H. (1979) J. Biol. Chem. 247, 9607-9613
11. Badin, J., Schubert, M., and Vouzas, M. (1955) J. Clin. Invest. 34, 1317-1323
12. Bassoumi, M. (1955) Ann. Rheum. Dis. 14, 298-209
13. Bolton, A. N., Serflying, M. W., and Simpson, W. F. (1957) J. Clin. Invest. 36, 1325-1332
14. Kerby, G. P. (1958) J. Clin. Invest. 37, 675-681
15. Schiller, S. (1958) Biochin. Biophys. Acta 28, 413-416
16. Friman, C., and Brunish, R. (1966) Proc. Soc. Exp. Biol. Med. 122, 590-601
17. Kannell, W. B., Castlewi, P. W., and McNamara, P. M. (1969) J. Atheroscler. Res. 9, 511-518
18. Goldstein, J. L., Hazazd, W. R., Schott, H. G., Birger, E. L., and Motulski, A. G. (1973) J. Clin. Invest. 52, 1533-1543
19. Skipi, V. P. (1972) in Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism (Nelson, G. L., ed) pp. 471-509, Wiley-Interscience, New York
20. Wagner, P. W., Roberts, B. L., White, H. J., and Read, R. C. (1973) Atherosclerosis 18, 83-91
21. Mancini, M., Rossi, G. B., Oriente, P., and Cali, A. (1965) Nature 207, 1206
22. Calatroni, A., Donnelly, P. V., and Di Ferrante, N. (1969) J. Clin. Invest. 48, 332-343
23. Berman, E. R. (1966) Nature 211, 640-641
24. Sajdera, S. W., and Hascall, V. C. (1969) J. Biol. Chem. 244, 77-87
25. Gerber, B. R., Franklin, E. C., and Schubert, M. (1960) J. Biol. Chem. 235, 9870-9875
26. Di Ferrante, N., Donnelly, P. V., and Berglund, R. K. (1971) Biochem. J. 124, 549-553
27. Constantopolous, G., Dukas, A. A., and Carroll, W. R. (1969) Anal. Biochem. 31, 59-70
28. Singh, J., Donnelly, P. V., Di Ferrante, N., Nichols, B. L., and Neides, P. (1974) J. Lab. Clin. Med. 84, 438-450
29. Yphantis, D. A. (1964) Biochemistry 3, 297-317
30. Tanford, C., Marler, E., Jury, E., and Davidson, E. A. (1964) J. Biol. Chem. 239, 4834-4840
31. Rosenberg, L., Pal, S., Beare, R., and Schubert, M. (1970) J. Biol. Chem. 245, 1112-1122
32. Brown, W. V., Levy, R. I., and Fredrickson, D. S. (1969) J. Biol. Chem. 244, 5687-5694

Downloaded from http://www.jbc.org/ by guest on March 22, 2020
33. Bragdon, J. H., Havel, R. J., and Boyle, E. (1956) J. Lab. Clin. Med. 48, 36–42
34. Jackson, R. J., and Gotto, A. M. (1972) Biochim. Biophys. Acta 286, 30–47
35. Pownall, H. J., Hu, A. S., and Smith, L. C. (1974) Circulation 50, Suppl. III; 260
36. Blumenkrantz, N., and Ashoe-Hansen, G. (1973) Anal. Biochem. 54, 484–489
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
38. McDougal, D. B., and Farmer, H. S. (1957) J. Lab. Clin. Med. 50, 489–498
39. Pownall, H. J., and Smith, L. C. (1973) J. Am. Chem. Soc. 96, 3136–3140
40. Soutar, A. K., Pownall, H. J., Hu, A. S., and Smith, L. C. (1974) Biochemistry 13, 2828–2836
41. Mohrson, L. M. (1971) Angiology 22, 105–174
42. Nakazawa, K., Murata, K., Izuka, K., and Oshima, Y. (1969) Jap. Heart J. 10, 280–296
The interaction of human plasma glycoaminoglycans with plasma lipoproteins.
Y Nakashima, N D Ferrante, R L Jackson and H J Pownall

J. Biol. Chem. 1975, 250:5386-5392.

Access the most updated version of this article at http://www.jbc.org/content/250/14/5386

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/250/14/5386.full.html#ref-list-1