Species Differences in Serum Glycoproteins of Man, Dog and Rabbit, by Paper Electrophoresis.* (22017)

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Paper electrophoresis provides a simple technic for the identification of serum proteins and stainable components bound to serum proteins. By application of the periodic acid-Schiff method for histochemical identification of various carbohydrate substances, Köiw and Gronwall first adapted this technic for the study of serum glycoproteins(1). The purpose of the present study was to establish a quantitative pattern of these components and their distribution in relation to serum proteins in normal human serum and in plasma from dogs and rabbits.

Procedure. Paper electrophoresis was performed according to a method described elsewhere(2,3). Filter paper strips were run in parallel for subsequent determination of proteins and glycoproteins. For the latter, .040 cc of human serum and rabbit plasma were employed, and .060 cc of dog plasma. In certain instances a third strip was added for determination of lipoproteins (Oil-red-O stain). The staining procedure employed for glycoproteins differed from that of Köiw and Gronwall in several steps. In preparation of the fuchsin sulfite (Schiff's reagent) 15 to 20 ml of concentrated hydrochloric acid rather than dilute acid were added as a final step. The paper strips were immersed in this solution under constant shaking for time periods varying usually between 10-15 minutes. The staining was continued until clear visibility of the purple bands was obtained without coloring of the background. The densitometric evaluation of the strips was performed immediately afterwards because of their tendency to darken after drying(4). The original color of the strips can be restored by brief immersion in the "sulfite rinse." Individual determinations were performed on serum from 9 normal human beings, six men and three women, ages 15 to 55 years, and clinically free of disease. Heparinized plasma from 6 normal male mongrel dogs and 8 normal male chinchilla rabbits were included in the study. The strips of human serum or animal plasma stained for glycoprotein (glycidograms) revealed 3 main components migrating with the mobility of alpha-1, alpha-2 and beta globulins. These appeared as distinct bands on the paper strips. The densitometric curves were readily separated into these 3 components. Gamma globulin was in our experience devoid of carbohydrate-staining material. Only faint amounts of material stained in the region of albumin; this fraction was therefore not included in evaluating the normal distribution pattern of the glycoproteins. Around the line of application of the serum a small amount of stainable material was usually visible which probably represents stainable substances adsorbed at the point of origin which showed no migration in the electrical field (0-fraction). Accordingly, the patterns were standardized only for the above-mentioned main components; alpha-1, alpha-2 and beta glycoproteins. Rough correlation was also observed in man between the total area of glycoprotein stained, measured by the planimeter, and the levels of total serum polysaccharide or hexosamine determined chemically.† This was noted despite the observation that different glycols may vary in their affinity for periodic acid(5,6). Even after filter paper strips had darkened on standing, densitometric evaluation after reimmersion in the "sulfite rinse" yielded curves and planimetric values identical to those obtained on initial examination. With strict adherence to this principle and with careful technic the method was found to be reproducible.

Results. In normal human serum the alpha-2 fraction was the largest (approximately

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TABLE I. Glycoprotein Distribution in Various Species.

Species	% of total stainable carbohydrate		
	Alpha-1	Alpha-2	Beta
Man	32.5	39.8	27.6
	24.6	41.1	34.2
	26.8	40.6	32.4
	33.9	39.7	26.4
	23.2	47.4	29.4
	31.7	35.0	33.3
	29.0	42.0	29.0
	31.4	42.9	25.7
	26.0	46.0	28.0
Mean	28.8 ± 3.8	41.6 ± 3.6	29.6 ± 3.1*
Dog	23.5	54.5	22.0
	27.0	52.5	20.5
	20.0	62.5	17.0
	21.0	52.5	26.5
	19.0	63.0	18.0
	29.0	51.5	19.5
Mean	23.2 ± 4.0	56.1 ± 5.3	20.6 ± 3.4*
Rabbit		92.5	7.5
		83.0	17.0
		85.0	15.0
		86.5	13.5
		90.0	10.0
		80.0	20.0
		82.0	18.0
		89.0	11.0
Mean		86.0 ± 4.3	14.0 ± 4.3*

* Statistical significance of differences of mean values for beta-glycoprotein: Man vs. dog, $p = .002$; man vs. rabbit, $p = < .0001$.

40%), while alpha-1 and beta were approximately equal, 30% each (Table I). In dogs, three glycoprotein bands were again identified; alpha-2 glycoprotein migrated somewhat more rapidly and was thereby closer in position to alpha-1 glycoprotein than in human serum (Fig. 1). The total quantity of stainable carbohydrate was less, necessitating the application of more plasma for a visible pattern (see procedure). Alpha-2 glycoprotein again was the main component, accounting, however, for even a greater percentage of the total stainable carbohydrate than in man (approximately 56%); alpha-1 and beta glycoprotein were present in about equal amounts but in lower proportion than in human serum (Table I). In rabbits, separation of alpha-1 and alpha-2 glycoprotein was not possible in many instances and, therefore, a single alpha-glycoprotein component was measured planimetrically. It was the major plasma glycoprotein component (approximately 86%) (Table I, Fig. 1). Beta glycoprotein was in this species considerably lower than in the other two.

No alteration of the normal plasma glycoprotein pattern of the rabbit was noted in 6 animals treated with corticosteroids [prednisone (Δ_1-Dehydrocortisone), cortisone, hydrocortisone] for 3-5 weeks nor in 3 rabbits in which hypercholesteremia had been induced.‡ In man, in several instances of multiple myeloma and liver disease a gamma glycoprotein band appeared in addition to the usual components and could be clearly separated from the 0-fraction.

Discussion. In this study a quantitative pattern of glycoprotein distribution in the serum (plasma) of man, rabbit and dog has been established by paper electrophoresis. To our knowledge, no previous report of such a study is available. Other investigators(1, 4, 7-9) have reported qualitative patterns of serum glycoproteins in normal man and in various disease states(1,4). Some of these patterns differed from those obtained in this study in that a gamma-glycoprotein was described in normal serum. The carbohydrate-staining fraction with slowest mobility (or perhaps no mobility) in our experience corresponded to that material adsorbed at the point of application of the serum (0-fraction). It did not move with the gamma globulin which under our experimental conditions migrated "backwards," *i.e.*, toward the cathode. In certain pathological states, however, a "true" gamma glycoprotein has been observed by others(1,4,7-9) and by ourselves. In all reports except that of Köiw and Gronwall, alpha-2 glycoprotein was the major component of the serum; in their graph, the largest glycoprotein peak was alpha-1.

The species examined showed distinctive patterns of glycoprotein distribution in a similar fashion to the species-specific protein and lipoprotein patterns described previously(2,3, 10,11). The glycoprotein distribution in man differed in a statistically significant manner from that obtained in dog and rabbit, with most marked divergence between man and rabbit. Previous examination of lipoproteins revealed a similar distribution pattern in man

‡ We are obliged to Dr. Chun-I Wang for providing these animals.

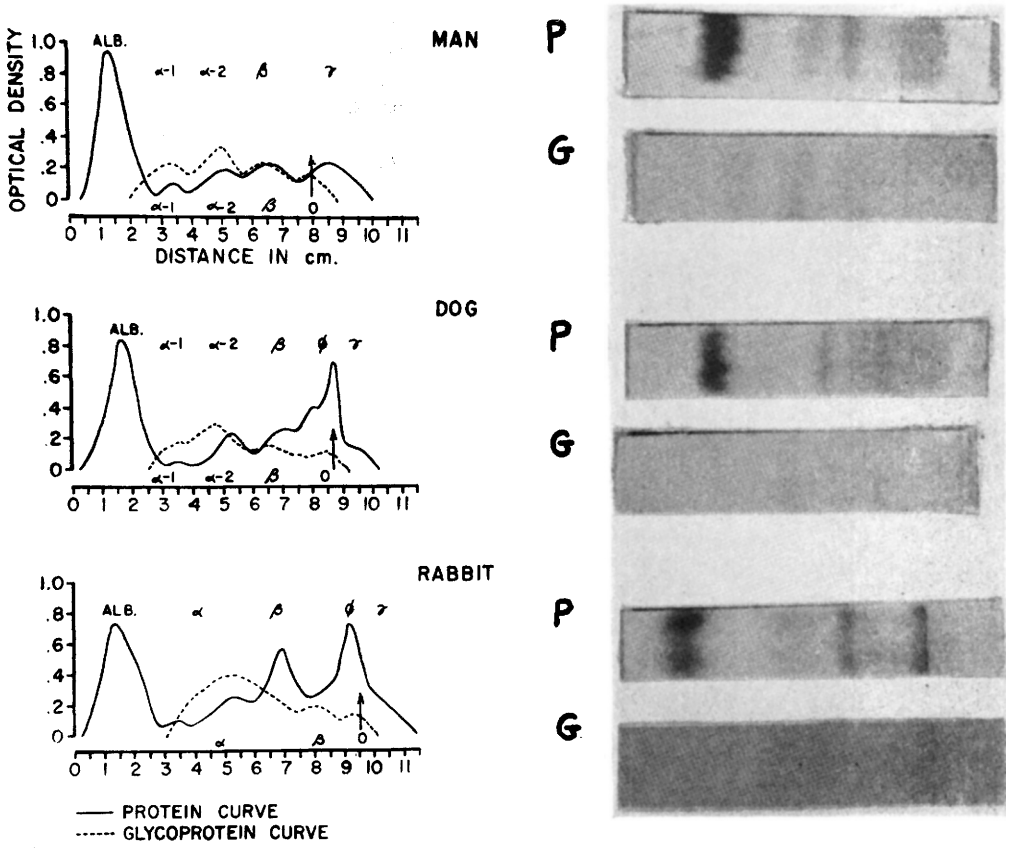

FIG. 1. Protein and glycoprotein electropherograms in man, dog and rabbit. On left are curves obtained by densitometric evaluation, on right are stained paper strips. P = Paper strip stained with amidoschwarz for protein. G = Paper strip stained with PAS for carbohydrate. Arrow in graphs and ruled line on paper strip represent the point of application of the serum (plasma). For details, see text.

and rabbit with regard to alpha and beta lipoprotein whereas in the dog the pattern was reversed. Thus, while species variations in these plasma components exist, they are not of parallel magnitude. Establishment of such normal patterns permits more detailed characterization of alterations in pathological states.

The technic of paper electrophoresis and subsequent periodic acid-Schiff stain did not yield values for distribution of serum glycoproteins comparable to those obtained by chemical analysis of plasma protein fractions (Cohn). The two procedures differed markedly and some discrepancy in results was not surprising; *e.g.*, alpha-1 glycoprotein appeared to be lower with the Cohn-fractionation technic. In addition, the analytical results of Cohn fractions differed with the author(12,13).

Summary. (1) By means of paper electrophoresis and subsequent carbohydrate staining by the periodic acid-Schiff method, quantitative evaluation of species glycoprotein patterns were established for man, dog and rabbit. (2) This procedure provides a simple, reproducible method for the quantitative study of serum glycoproteins which may be correlated with simultaneous determination of proteins and lipoproteins. (3) Carbohydrate was bound to alpha-1, alpha-2 and beta-globulins, with the major portion corresponding to the alpha globulins. Gamma glycoprotein was absent in normal serum in man. (4) No alterations were observed in rabbits after administration of adrenal cortical ster-

oids nor in experimentally-induced hypercholesteremia.

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ERRATUM

In Vol. 88, 1955, article on rapid virulence test in diphtheria, page 369, line 8, 0.0045% should read 0.033%.