

(13) might in part have been due to contamination of the insulin with HGF.

Summary. HGF increases GFR and RPF consistently but transiently in the dog, does not affect FF, at times decreases Tm_G . Tolerance does not develop during long term daily HGF administration nor does this regimen increase the basal renal functions.

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Electrophoretic Studies of Bovine Serum. IV. Differences in Serum Glycoproteins Due to Age and Sex.* (25369)

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Presence of protein-bound carbohydrates of the hexose variety in serum proteins has long been recognized, although very little is known concerning their physiological significance. Only recently has attention been focused on the clinical significance of these glycoproteins in human medicine(1,2) and to a limited extent in experimentation with small laboratory animals(3-5) and swine(6). It has been demonstrated that concentrations of serum glycoproteins are abnormally elevated in many pathological and physiological conditions(7-9) either as a result of tissue destructive(7,9) or proliferative processes(8,9). Limited data on glycoproteins in parasitic infestations in small laboratory animals have been published(10). In these cases significant alterations were not found. A study of serum glycoproteins of the bovine species has not been reported. Mention has been made that the albumin, alpha-, beta-, and gamma-

globulins have some glycoprotein associated with each fraction as determined by paper electrophoresis(11). Due to our interest in various diseases of cattle, and the possible significance of serum glycoproteins in formulation of mechanisms of disease processes, an investigation was made of glycoproteins in normal bovine serum. This report presents information on the relative and absolute concentrations of glycoproteins associated with serum proteins. Values are also given with respect to age and sex differences.

Materials and methods. *Cattle:* Serum glycoproteins from 3 dairy herds of single and mixed breeds managed under southern Louisiana conditions were studied. Two herds consisted of 232 females ranging in age from 1 month to 13 years. The third herd consisted of 35 stud bulls ranging in age from 2 to 15 years. All animals were considered to be normal and appeared to be free from any apparent physiological or pathological disturbances. Blood was collected individually in early

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TABLE I. Statistical Summary of Relative Concentrations of Glycoproteins Bound to Components of Normal Bovine Serum Obtained from Various Age Groups.

Mean % conc. and stand. dev. of glycoproteins bound to serum fractions					
Age group (mo)	No. of samples	Albumin	Globulins		
			α	β	γ
>24	160	3.3 $\pm$.3	50.0 $\pm$.7	25.0 $\pm$.5	21.7 $\pm$.5
18-24	28	3.5 $\pm$.6	53.0 $\pm$ 1.3	26.0 $\pm$ 1.5	17.6 $\pm$ 1.1
12-18	26	2.6 $\pm$.8	55.4 $\pm$ 1.6	24.1 $\pm$ 1.1	17.9 $\pm$ 1.0
9-12	8	3.7 $\pm$ 1.5	62.5 $\pm$ 3.1	17.7 $\pm$ 3.6	16.1 $\pm$ 2.7
6-9	14	6.4 $\pm$ 1.4	53.0 $\pm$ 2.6	20.9 $\pm$ 1.2	19.8 $\pm$ 2.4
3-6	20	5.0 $\pm$ 1.3	58.0 $\pm$ 2.6	21.4 $\pm$ 1.2	15.6 $\pm$ 1.5
<3	11	3.9 $\pm$ 1.2	63.4 $\pm$ 1.7	19.4 $\pm$ 1.0	13.4 $\pm$ 1.5
F values*		1.95	9.12†	5.60†	7.11†

* Analysis of variance.

† Significant at .01 level of probability.

spring. Each serum sample was stored at -22°C until analyzed for glycoproteins, using paper electrophoretic techniques and chemical determinations. Our unpublished data have shown that freezing and thawing 1 time does not alter glycoprotein content of bovine serum. *Electrophoresis*: Each serum sample was applied to a paper strip 4 times in 0.01 ml amounts giving total volume of 0.04 ml. This volume of serum was the minimum sufficient to yield measurable amounts of glycoprotein when using a photoelectric densitometer-integrator. Volumes above 0.04 ml produce smearing of proteins that are separated on paper and are not suitable. Proteins were separated in barbital buffer of pH 8.6 and 0.075 ionic strength. A current of 0.63 milli-ampere/strip was applied for 17 hours at 23°C . Then the strips were dried and fixed at 125°C for 30 minutes. *Staining and determination of glycoproteins*: All strips were stained with periodic acid Schiff reagent as described by Köiw and Grönwall(12). After drying at 23°C the strips were scanned for relative % composition of glycoproteins by

using a densitometer-integrator equipped with 550 $m\mu$ interference filters. To convert these relative values of each glycoprotein associated with a specific serum fraction to absolute weights, it was necessary to determine total content of glycoproteins present. The tryptophan method of Shetlar, *et al.*(13) was used in analyses of duplicate samples of serum.

Results. Mean values and standard deviations of the relative concentrations of protein fractions are shown in Table I. Analysis of variance showed that the differences in relative amounts of albumin among 267 animals in 7 age groups were not statistically significant. It was evident that significant differences occurred in glycoproteins in that younger animals possessed relatively more alpha-globulin glycoprotein and less beta- and gamma-globulin glycoprotein than older animals.

Results obtained from a compilation of data on absolute concentrations of serum glycoproteins are presented in Table II. The data show that there were no significant differences in weights of glycoproteins associated with the

TABLE II. Statistical Summary of Absolute Concentrations of Glycoproteins Bound to Components of Normal Bovine Serum Obtained from Various Age Groups.

Mean mg % conc. and stand. dev. of glycoproteins bound to serum fractions						
Age group (mo)	No. of samples	Albumin	Globulins			Total
			α	β	γ	
>24	160	3.9 $\pm$.4	60.7 $\pm$ 1.1	30.4 $\pm$.6	26.3 $\pm$.7	121.3 $\pm$ 1.3
18-24	28	3.7 $\pm$.7	58.0 $\pm$ 2.0	28.3 $\pm$ 1.2	18.9 $\pm$ 1.1	108.9 $\pm$ 1.7
12-18	26	2.7 $\pm$.8	62.5 $\pm$ 2.8	26.8 $\pm$ 1.3	20.0 $\pm$ 1.3	112.0 $\pm$ 2.8
9-12	8	3.7 $\pm$ 1.5	63.6 $\pm$ 3.0	18.4 $\pm$ 3.7	16.4 $\pm$ 2.7	102.2 $\pm$ 2.9
6-9	14	6.9 $\pm$ 1.4	58.6 $\pm$ 3.7	23.2 $\pm$ 1.8	21.9 $\pm$ 2.1	110.6 $\pm$ 5.1
3-6	20	4.9 $\pm$ 1.3	59.5 $\pm$ 3.2	21.8 $\pm$ 1.2	15.9 $\pm$ 1.5	102.0 $\pm$ 2.1
<3	11	3.7 $\pm$ 1.1	58.9 $\pm$ 1.4	18.1 $\pm$ 1.1	12.5 $\pm$ 1.5	93.1 $\pm$ 1.5
F values*		1.50	.520	11.59†	12.88†	14.63†

* Analysis of variance.

† Significant at .01 level of probability.

TABLE III. Statistical Summary of Relative Concentrations of Glycoproteins Bound to Components of Normal Male and Female Bovine Serum from a Similar Age Group (2 Years and Older).

Sex and No. of samples	Albumin	Mean % conc. and stand. dev. of glycoproteins bound to serum fractions			
		α	β	γ	
♂ 35	3.7 $\pm$.6	43.0 $\pm$ 1.0	30.2 $\pm$ 1.0	23.1 $\pm$ 1.0	
♀ 125	3.2 $\pm$.4	51.9 $\pm$.7	23.6 $\pm$.4	21.2 $\pm$.6	
F values*	.137	36.83†	48.53†	1.73	

* Analysis of variance.

† Significant at .01 level of probability.

TABLE IV. Statistical Summary of Absolute Concentrations of Glycoproteins Bound to Components of Normal Male and Female Bovine Serum from a Similar Age Group (2 Years and Older).

Sex	No. of samples	Mean mg % conc. and stand. dev. of glycoproteins bound to serum fractions				
		Albumin	α	β	γ	Total
♂ 35	35	4.6 $\pm$.8	53.6 $\pm$ 1.5	37.7 $\pm$ 1.4	29.9 $\pm$ 1.5	124.8 $\pm$ 2.3
♀ 125	125	3.8 $\pm$.4	62.7 $\pm$ 1.2	28.3 $\pm$.6	25.6 $\pm$.8	120.4 $\pm$ 1.5
F values*		.794	13.27†	49.09†	4.31†	2.35

* Analysis of variance.
level of probability.

† Significant at .05 level of probability.

‡ Significant at .01

albumin and alpha-globulin fractions between age groups. It appeared that younger animals possessed significantly less total glycoprotein and beta- and gamma-globulin glycoprotein than older animals.

Analysis of variance between 35 males and 125 females, all 2 years of age and over, showed statistically significant differences in relative amounts of alpha- and beta-globulin glycoproteins. Table III indicates that males possessed less alpha-globulin glycoprotein and more beta-globulin glycoprotein than females. Significant sex differences did not occur in the other fractions.

Table IV presents data on sex differences in absolute weights of serum glycoproteins. Males possessed less alpha-globulin glycoprotein and more beta-globulin and gamma-globulin glycoproteins than females. There were no significant sex differences in weights of albumin glycoprotein and total glycoprotein.

Discussion. The reasons for differences in concentrations of glycoproteins associated with various serum fractions are not definitely known and have been the subject of speculation(9,14). Winzler(9) proposed that due to

the multiplicity of serum glycoproteins it is possible that the different components are concerned with divergent physiologic processes. Possibly, these differences occur as a direct result of the maturation process of the calf into an adult when hormonal and other influences are in operation.

Reasons for lowered amounts of glycoproteins in serums of young animals are conjectural. Shetlar, *et al.*(14) reported that human fetal serum contains less polysaccharide than serum from adults. Younger bovine ani-

mals possess less total serum proteins(15) and possibly cannot bind as much glycoprotein.

The possibility that the amounts of serum proteins influence the amounts of glycoproteins bound, that the differences observed may occur as a result of lactation, hormonal influences between sexes, sexual maturity, metabolic rates, and other diverse physiological stimuli are subjects for further investigation.

Summary. Differences found in glycoproteins of bovine serum with respect to age and sex are presented.

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Effect of Sodium Chloride Concentration on Staphylococcal Agglutination.* (25370)

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Since it has been shown that concentration of salts affects direct bacterial agglutination (1,2), it was decided to evaluate the degree of this effect in macroscopic staphylococcal agglutination. Spontaneous agglutination, the quantity of agglutination as measured by highest agglutination titer and the quality of agglutination as determined both by reproducibility and sharpness of endpoint and gross particulation of aggregates were observed.

Method and materials. Four different strains of hemolytic coagulase positive staphylococci representing the following phage types were used[†]

Strain	Phage type
a	29
b	81
c	4, 6, 29, 29A, 31, 79, 44, 52, 52A, 71, 3A, 42E, 44A, 73, 75, 51, 81
d	51, 42B, 81

Antisera were obtained from rabbits immunized intravenously with these strains grown

in broth and killed with 0.25% phenol. Human sera were obtained from adult male volunteers. The term agglutination reaction is restricted to visible (second) stage of agglutination and precipitation as determined macroscopically, and does not imply consideration of combination or dissociation of antibody and antigen. Antigen concentration was that giving a density equal to that of the Standard Typhoid Vaccine (1000 million organisms/ml) supplied by Division of Laboratories and Research, N. Y. State Dept. of Health. Agglutination reactions were carried out as follows: Serial 2-fold dilutions of serum were made in standard agglutination tubes (½ x 3 inch) with the desired concentration of salt solution. One half ml was left in each tube. The organisms were kept in frozen skim milk until used. The original or first subculture from skim milk was used as antigen. After overnight incubation on rabbit agar, the growth was removed with a sterile cotton swab from surface of plate and suspended in desired concentration of sodium chloride solution. After the suspension was filtered through a small cotton plug, it was adjusted to a density twice that of optimal antigen concentration. The antigen suspension was then added to the serial 2-fold dilutions of serum in a ratio of 1:1 thus giving final volume of one milliliter. The mixture was incubated in water bath at

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