

## GENETIC POLYMORPHISM IN HUMAN GLYCINE-RICH BETA-GLYCOPROTEIN\*,†

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(Received for publication 18 August 1971)

Glycine-rich  $\beta$ -glycoprotein (GBG)<sup>1</sup> (1) is a 6.2S globulin which, on storage of serum, slowly converts to two fragments. The more basic fragment, glycine-rich  $\gamma$ -glycoprotein (GGG) (2) is probably identical to  $\beta_2$ -glycoprotein II (3), originally described as a native serum protein. The antigenic determinants on the two fragments appear to be distinct in that antiserum to GGG produces a reaction of identity between GBG and GGG in immunoelectrophoresis and no reaction with the acidic fragment (2). On the other hand, antiserum to GBG produces reactions with GBG and both fragments with spurring of the GBG arc over both GGG and glycine-rich  $\alpha$ -glycoprotein (GAG) (1). Rapid conversion of GBG has been observed on incubation of whole serum with antigen-antibody precipitates, zymosan, or endotoxin at 37°C (4).

This report is concerned with evidence for genetically controlled polymorphism in GBG. The nature of the polymorphism is such that certain conclusions concerning the structure of the molecule have been drawn. A preliminary brief report of these studies has been published (4).

### *Materials and Methods*

*Preparation of Antisera.*—Antiserum to GBG was prepared in rabbits as described previously (1). This antiserum reacted with GAG, as well as with GBG and GGG.

Antiserum to rabbit serum proteins was produced in a goat by multiple intracutaneous injections of 0.1 ml whole rabbit serum emulsified in complete Freund's adjuvant. This antiserum reacted with rabbit immunoglobulins (as well as other rabbit serum proteins) when tested in immunoelectrophoresis against whole rabbit serum.

*Agarose Gel Electrophoresis.*—Electrophoresis in agarose gel was performed either as described by Laurell and Niléhn (5) for short separations or in a modified apparatus for pro-

\* Supported by Grant AM 13855 from the National Institutes of Health.

† Presented in part at the Fourth International Complement Workshop in Baltimore, Md., January, 1971, and the First International Congress of Immunology, Washington, D. C., August, 1971.

<sup>1</sup> *Abbreviations used in this paper:* GAG, glycine-rich  $\alpha$ -glycoprotein; GBG, glycine-rich  $\beta$ -glycoprotein; GGG, glycine-rich  $\gamma$ -glycoprotein; LDH, lactate dehydrogenase.

longed electrophoresis (6). Veronal buffer at pH 8.6 and ionic strength 0.05 or 0.025 with 0.0018 M calcium lactate was used for gel and electrode buffers.

*Immunofixation Electrophoresis.*—Immunofixation after agarose gel electrophoresis was carried out as described previously (7), except that faint patterns were intensified by a second immunofixation with antiserum to rabbit serum proteins.

*Antigen-Antibody-Crossed Electrophoresis.*—The technique described by Laurell for antigen-antibody-crossed electrophoresis (8) was used. Faint patterns were intensified by immunofixation with antiserum to rabbit immunoglobulins as described above.

*Serum Samples.*—Serum was obtained by centrifugation from venous blood allowed to clot for about 1 hr at room temperature. The serum was kept at  $-80^{\circ}\text{C}$  and thawed immediately before analysis.

For analysis of the conversion products, GAG and GGG, fresh serum or fresh serum stored at  $-80^{\circ}\text{C}$  was incubated for 60 min at  $37^{\circ}\text{C}$  with antigen-antibody-specific precipitates (2 mg/ml of serum) or zymosan (2 mg/ml of serum) before electrophoresis.

*Population Studies.*—Samples were obtained from unrelated individuals of the major racial groups.

*Family Studies.*—The familial relationships were corroborated in all cases by analysis of red cell antigen and other serum protein genetic types. Several of the larger families were used in earlier studies (6, 9). Mother-cord serum pairs were obtained at the time of delivery.

## RESULTS

*Common GBG Patterns in Random Sera.*—On immunofixation after prolonged agarose gel electrophoresis, GBG in most sera from all racial groups formed one of the three patterns shown in Fig. 1. As can be seen, patterns I and III consisted of at least four equally spaced components and pattern II of at least five such components. Patterns I and III were similar with respect to the distribution of the component band densities. They differed in that all components in pattern III appeared shifted one electrophoretic "position" toward the anode with respect to the components in pattern I. Pattern II appeared to represent a superimposition of patterns I and III and could, in fact, be reproduced by mixing equal volumes of sera of type I and III.

*Proposed Genetic Basis for the Common GBG Patterns in Random Sera.*—If one assumes that the fast common pattern is the product of one allele, ( $Gb^F$ ), and the slower pattern is the product of another allele, ( $Gb^S$ ), then the donor of serum III would be  $Gb^{FF}$  and the donor of serum I would be  $Gb^{SS}$ , whereas the donor of serum II would be heterozygous or  $Gb^{FS}$ . This explanation predicts that if there are other alleles at the Gb locus producing variants of different electrophoretic mobility than Gb S or Gb F, the common gene product would look like Gb S or Gb F, but not Gb FS.

*Rare GBG Variants.*—In some sera from Negroes, the GBG pattern was unusual in that it contained a complex of components shifted two electrophoretic positions toward the anode with respect to Gb F. The common area in such sera was occupied by Gb F or Gb S, but never Gb FS (Fig. 2). In a few sera from Caucasians, there was an unusual complex of components which migrated two electrophoretic positions toward the cathode with respect to

Gb S. Similarly, in these sera the rare variant was accompanied by Gb S or Gb F, but not both (Fig. 2). The rare fast variant was designated  $F_1$  and the rare slow variant,  $S_1$ . Since it seemed likely for the reasons given that the genes producing  $F_1$  and  $S_1$  were allelic to  $Gb^F$  and  $Gb^S$ , they were called  $Gb^{F_1}$  and  $Gb^{S_1}$ .

*Evidence for Autosomal Codominant Inheritance in the Gb System.—*

*Family studies:* The results of examining GBG patterns in 27 families are

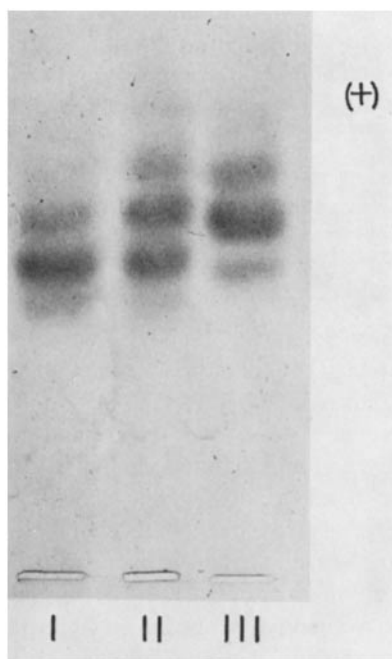


FIG. 1. GBG patterns in whole sera developed by prolonged electrophoresis in agarose gel at pH 8.6 followed by immunofixation with specific antiserum. Gb types (as described in text) are: I = SS, II = FS, III = FF.

given in Table I. In no instance was an unexpected type found among the offspring of parents of various Gb types. There was male-to-male transmission of types and many males were Gb heterozygotes.

*Population studies:* Gb gene frequencies in random unrelated individuals are given in Table II. There were no important differences in frequencies between males and females within each racial group. Gene frequencies differed significantly from racial group to racial group, however, as is shown in Table II. Observed Gb types and those expected from the Hardy-Weinberg equilibrium agreed very closely as can be seen in Table III.

Of the ten Gb phenotypes predicted by the presence of four alleles, seven have been observed: SS, FS, FF,  $F_1F$ ,  $F_1S$ ,  $FS_1$ , and  $SS_1$ .

*Failure of GBG to Cross the Placenta.*—Gb types in maternal-cord serum pairs were compared (Table IV). It is apparent that GBG does not cross the placental barrier in either direction since over half the pairs showed differences in type. There was no evidence for admixture in any of these sera.

The mean GBG concentration in the maternal sera was significantly elevated ( $P < 0.01$ ) compared with normal controls (mean 34 mg%), whereas the

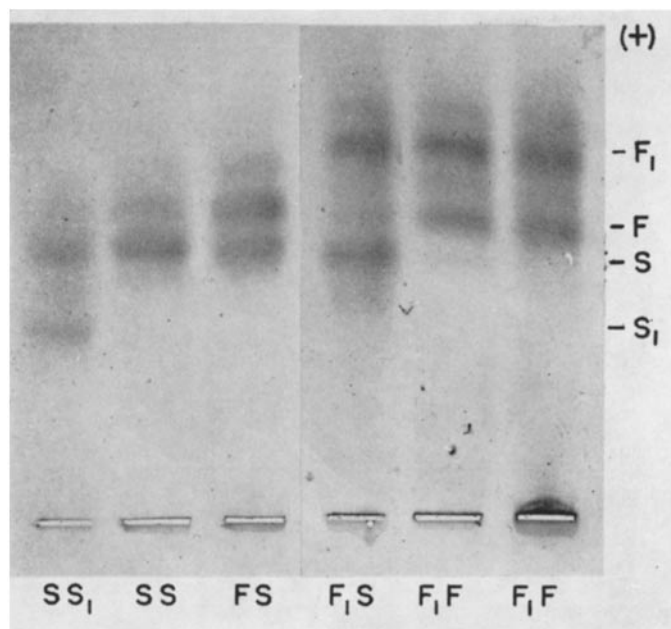


FIG. 2. GBG patterns in whole sera developed as described in Fig. 1. The rare variants Gb  $F_1$  and Gb  $S_1$  are shown in comparison with Gb FS and Gb SS. Note that in the sera with rare variants, Gb F or Gb S (but not both) is found.

mean cord level was less than a third of that in maternal sera. The cord levels were also significantly lower than normal ( $P < 0.01$ ).

*The Number, Position, and Concentration Relationship of GBG Electrophoretic Components.*—GBG in all sera examined consisted of at least four components on immunofixation electrophoresis, even in sera from Gb homozygotes. On antigen-antibody-crossed electrophoresis (Fig. 3) of Gb FF and Gb SS sera, it could be shown that there was a fifth component anodal to the others. The distance between adjacent components was the same throughout the pattern. The approximate ratio of peak heights (from anode to cathode) was 1:5:12:25:4 in both homozygous patterns, Gb FF and Gb SS. Because no attempt at

curve resolution was made and because it was not possible to accurately measure the lowest peak, these ratios must be regarded as crude.

*Reflection of Gb Polymorphism in the Fragments, GGG and GAG.—*

(a) GBG of various types was partially converted to GGG and GAG by incubation of serum with antigen-antibody precipitates or zymosan. The sera were then examined by immunofixation with anti-GBG after agarose gel electrophoresis. In Fig. 4, the GBG and GGG areas of such converted sera are

TABLE I  
*Gb Types in Families*

Parental types	No. of families	Offspring types*			
		FF	FS	SS	F <sub>1</sub> S
FS × SS	11	0 (0)	14 (14.5)	15 (14.5)	0 (0)
FS × FS	3	0 (1.5)	6 (3)	0 (1.5)	0 (0)
FF × SS	1	0 (0)	1 (1)	0 (0)	0 (0)
FS × FF	2	4 (2)	1 (2)	0 (0)	0 (0)
SS × SS	9	0 (0)	0 (0)	24 (24)	0 (0)
F <sub>1</sub> F × SS	1	0 (0)	0 (0.5)	0 (0)	1 (0.5)

\* Expected numbers are given in parentheses.

TABLE II  
*Calculated Gb Gene Frequencies among Random Unrelated Individuals*

	No. tested	$Gb^S$	$Gb^F$	$Gb^{F_1}$	$Gb^{S_1}$
Negro	127	0.437	0.512	0.051	—
Caucasian	158	0.709	0.278	—	0.013
Oriental	86	0.890	0.110	—	—

shown. Although the electrophoretic separation is insufficient to resolve all the components of GBG in these sera, the Gb types were distinguishable. It is also seen that whereas GGG from Gb FF, SS, and FS formed a single major band of a common electrophoretic mobility, GGG from Gb F<sub>1</sub> and S<sub>1</sub> was very different. Thus, the variant structural features of these rare variants but not those of the common variants, Gb F and S, were carried by GGG.

(b) When GAG in sera of various Gb types was examined by antigen-antibody-crossed electrophoresis, it was found that GAG from Gb FF or SS gave patterns with a single major peak with at least two minor peaks (Fig. 5).

TABLE III  
*Observed and Expected\* Gb Types in Random Unrelated Individuals*

Race	No.		SS	FS	FF	F <sub>1</sub> F	F <sub>1</sub> S	FS <sub>1</sub>	SS <sub>1</sub>
Negro	127	(Observed)	24	56	34	6	7	0	0
		(Expected)	24.2	56.8	33.3	6.6	5.7	—	—
Caucasian	158	(Observed)	78	64	12	0	0	0	4
		(Expected)	79.4	62.3	12.2	—	—	1.1	2.8
Oriental	86	(Observed)	68	17	1	0	0	0	0
		(Expected)	68.0	16.9	1.0	—	—	—	—

\* Based on the Hardy-Weinberg equilibrium  $(p + q)^2$  or  $(p + q + r)^2$  where p, q, and r represent observed gene frequencies.

TABLE IV  
*GBG Type and Concentration in Paired Maternal-Cord Sera*

Pair	Maternal		Cord	
	Type	GBG	Type	GBG
		<i>mg/100 ml</i>		<i>mg/100 ml</i>
1*	FS	54	SS	8
2	FS	34	FS	7
3*	FS	46	SS	15
4	FS	61	FS	19
5	FS	32	FS	13
6*	FS	67	FF	22
7	SS	42	SS	10
8	FS	39	FS	13
9*	FS	56	SS	13
10	FS	39	FS	13
11	FS	34	FS	8
12*	FS	42	SS	8
13*	FF	53	FS	13
14*	FS	36	SS	2
15	FS	57	FS	22
16*	SS	42	FS	15
17	FS	45	FS	10
18*	FS	31	SS	15
19*	FF	22	FS	13
20	SS	56	SS	16
21*	FS	35	SS	8
22*	FS	53	SS	12
23*	FF	20	FS	10
24*	FS	31	SS	1
25*	FS	16	SS	10
Mean ( $\pm$ sd)		42 $\pm$ 13.1		12 $\pm$ 5.1

\* Maternal and cord types different.

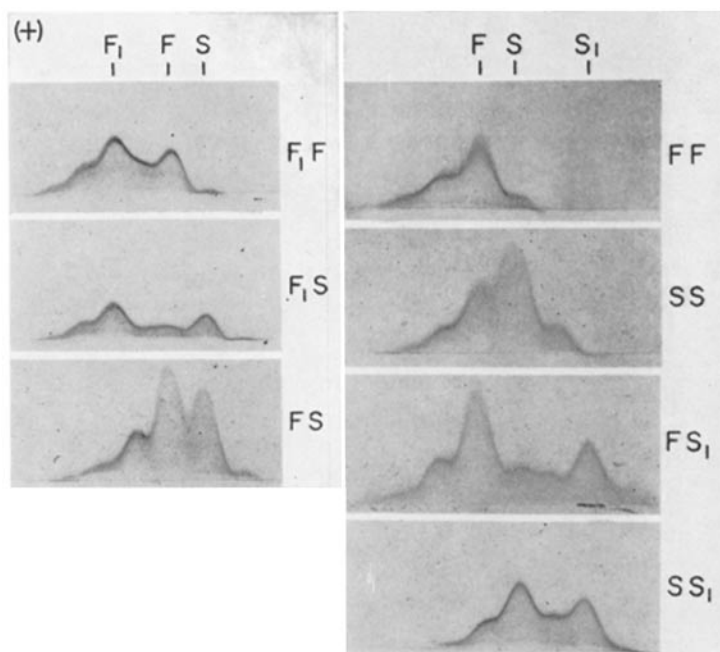


FIG. 3. Antigen-antibody-crossed electrophoresis patterns of the seven known Gb phenotypes. Electrophoresis in the first direction was performed with the anode at the left and in the second, into anti-GBG, the anode was at the top. Gb types are given to the right of each pattern, and the positions of the main components are given at the top of the figure.

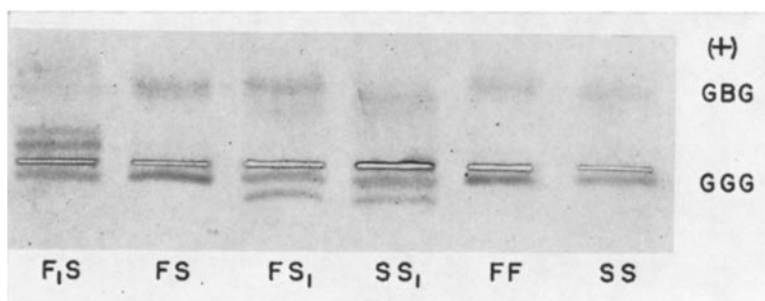


FIG. 4. GBG and GGG patterns in sera treated with antigen-antibody aggregates. Electrophoresis was carried out in agarose gel at pH 8.6 and patterns were developed with anti-GBG. Despite the short separation, the Gb types (given below each slot) are discernible.

GAG from Gb FS, on the other hand, consisted of two major peaks and at least two additional minor peaks. These findings suggested that the structural differences between Gb F and Gb S resided in the GAG fragment of GBG. The GAG fragments from Gb SS<sub>1</sub> or F<sub>1</sub>S gave antigen-antibody-crossed

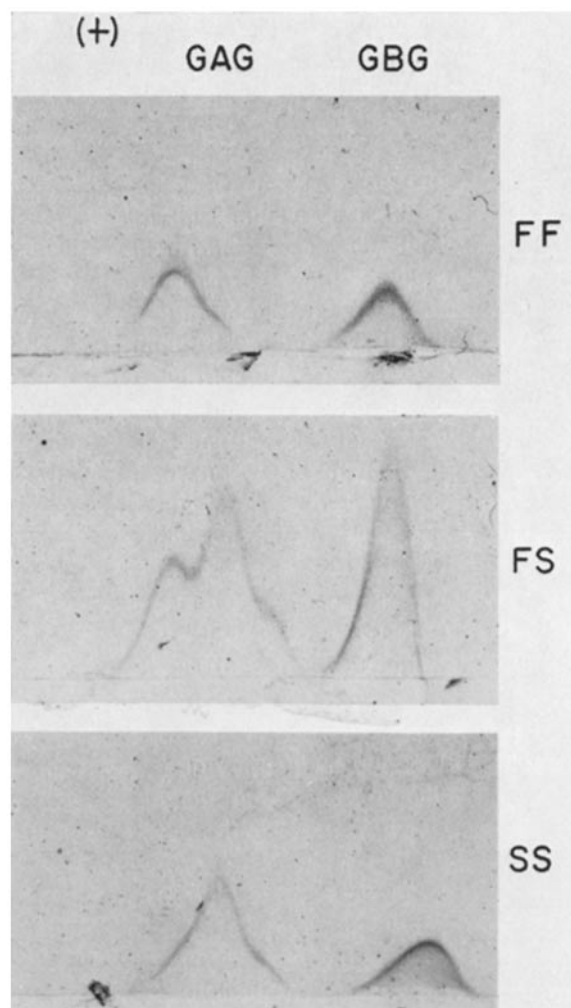


FIG. 5. GAG patterns in sera treated with antigen-antibody aggregates and analyzed by antigen-antibody-crossed electrophoresis. Gb types are not distinguishable.

electrophoresis patterns indistinguishable from those of GAG from Gb SS, whereas GAG fragments from Gb FS<sub>1</sub> or F<sub>1</sub>F were the same as those from Gb FS.

#### DISCUSSION

The observations presented establish that GBG is highly polymorphic and that this polymorphism is genetically determined. Some aspects of this polymorphism give indications of the gross structure of the GBG molecule.

The presence of five equally spaced components of GBG after electrophoresis of sera from Gb homozygotes strongly suggests that the molecule consists of randomly associated subunits of different net charge at pH 8.6. In analogy to lactate dehydrogenase (LDH) for which this phenomenon was first clarified by Markert and his coworkers (10-12), let us designate the more anodal subunit B and the more cathodal A. Then, the electrophoretic bands of GBG most probably have the composition (from anode to cathode)  $B_4$ ,  $AB_3$ ,  $A_2B_2$ ,  $A_3B$ , and  $A_4$ . Since the GBG bands occur only at fixed intervals and no extra bands have been observed within these intervals in any sera, all individuals thus far examined are apparent homozygotes for the genes controlling the structure of the A and B subunits.

The homozygous Gb patterns FF and SS differ from proteins such as LDH

TABLE V  
*Theoretical Tetramer Ratios for Various Relative Inputs of Two Subunits, A and B*

Input ratio A/B	Band 5 $A_4$	Band 4 $A_3B$	Band 3 $A_2B_2$	Band 2 $AB_3$	Band 1 $B_4$
1	1	4	6	4	1
1.5	5.1	13.5	13.5	6	1
1.6	6.5	16.4	15.4	6.4	1
1.7	8.3	19.6	17.3	6.8	1
1.8	10.5	23.3	19.4	7.2	1
1.9	13	27.4	21.7	7.6	1
2	16	32	24	8	1
3	81	108	54	12	1
4	256	256	96	16	1
5	625	500	150	20	1
R	$R^4$	$4R^3$	$6R^2$	$4R$	1

artificially produced from mixtures of equal amounts of A and B subunits (12) or naturally occurring heterozygous rhesus thyroxine-binding prealbumin (13, 14) in that the Gb patterns are clearly asymmetrical. Similar, if not identical, asymmetry is seen in each of the Gb gene products. The concentration ratio of LDH tetramers produced from equal amounts of A and B subunits is 1:4:6:4:1, and this is probably also true of the rhesus PA FS heterozygote. In both cases, the "inputs" of the two different kinds of subunits are equal. Where the inputs are unequal, the concentration ratios of tetramer components is determined by the A:B ratio, R, according to the relationship 1:4R:6R<sup>2</sup>:4R<sup>3</sup>:R<sup>4</sup> (from anode to cathode).

Table V gives the component ratios for various values of R. From the observed relationships of peaks in the homozygous Gb patterns, FF and SS, of Fig. 3, it is clear that R lies between 1.5 and 3, i.e., there is apparently between 1.5 and 3 times as much A subunit input as B input for random tetramer

formation. As judged from the patterns of Gb FF and SS on antigen-antibody-crossed electrophoresis (Fig. 3), peaks 2 and 5 (numbering from anode to cathode) are approximately equal in height. From Table V it is evident that this relationship holds when the A to B input ratio is around 1.6. On the assumption that peaks 2 and 5 are exactly equal,  $R^4 = 4R$  or  $R = \sqrt[4]{4} = 1.59$ . Most reasonably, this represents different rates of synthesis by the genes controlling the A and B subunits. While it is possible that differential rates of catabolism for the different GBG tetramer components account for the peak asymmetry, this appears less likely.

The genetically determined electrophoretic mobility shifts that define the Gb polymorphism must reside in yet a third moiety of the GBG molecule. Genetically controlled variations in the charge of this third or C moiety can

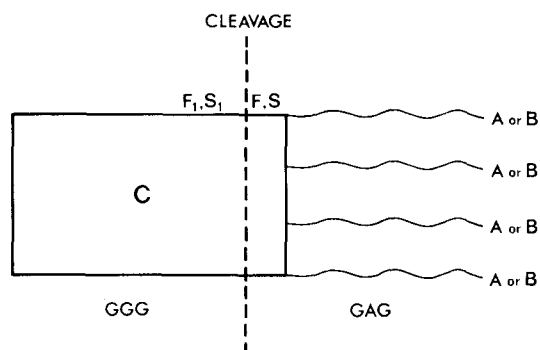


FIG. 6. Schematic model of the GBG molecule based on evidence obtained in the study of the genetic polymorphism.

explain the Gb types if it is assumed that the ratio of C to  $(A, B)_4$  tetramer is the same for all GBG molecules.

If the preceding considerations are correct, the observations that the variant residues of Gb  $F_1$  and  $S_1$  are found in the GGG portion of GBG, whereas those for Gb F and S are in the GAG portion of the molecule, suggest that fragmentation as the result of complement or properdin activation occurs through the C moiety. Because GGG is single (or at most double) banded and because GAG appears to consist of multiple components reminiscent of the parent molecule, a reasonable postulated structure for GBG is that shown in Fig. 6.

An interesting corollary of this scheme is that the genes for the rare variants Gb  $S_1$  and  $F_1$  may have arisen from  $Gb^s$ , since GAG fragments from GBG of types  $F_1$ ,  $S_1$ , and S are electrophoretically indistinguishable. Furthermore, since Gb F and Gb S differ by only one electrophoretic position, if one assumes that a single charge difference is responsible for this difference, then the ratio of C to  $(A, B)_4$  would be 1.

It is clear that these structural conclusions, drawn solely from considerations of the genetic polymorphism in GBG, require careful substantiation from more direct analyses.

Convincing evidence has recently been obtained<sup>2</sup> that purified preparations of GBG have properdin Factor B activity (15). It had been demonstrated some time ago that the action of properdin on the "classical" third component of complement (C3) required two protein cofactors, Factor A which was hydrazine sensitive, but not C4 (16), and Factor B which was very heat-labile, but not C2 (15). In a patient with increased susceptibility to infection and congenital hypercatabolism of C3 (17-19), no serum GBG was detectable; properdin activity and properdin Factor B activity<sup>3</sup> were absent despite a normal concentration of properdin protein determined immunochemically. In addition, proteolytic activity for GBG was found in his serum (20). Small amounts of normal serum added to his serum suppressed this GBGase activity completely, indicating that this serum lacked a normal GBGase inhibitor. If properdin factor B and GBG are identical, the question of the relationship of the properdin system to host resistance to infection assumes new relevance.

A recent exchange of antisera with Dr. Otto Götze has established that antibody to purified C3 proactivator (21) has the same antigenic specificity as anti-GGG or anti- $\beta_2$ -glycoprotein II. Preparations of purified C3 proactivator similarly had properdin Factor B activity (22).

#### SUMMARY

Extensive polymorphism of glycine-rich  $\beta$ -glycoprotein (GBG) was found in human sera. In all instances, GBG consisted of at least five components on electrophoresis. Patterns were such that they provided evidence for four alleles (at a locus designated Gb) which were expressed as autosomal codominant traits.  $Gb^s$  and  $Gb^F$  were found in all populations but with different frequencies,  $Gb^{F1}$  was found in Negroes, and  $Gb^{s1}$  was found in Caucasians.

From electrophoretic studies of GBG, evidence was obtained that suggested that the GBG molecule was a tetramer consisting of A and B subunits in a proportion of about 1.6:1. The genetically controlled differences in GBG embodied in the Gb system indicated the presence of a third moiety of the molecule (C), possibly a polypeptide subunit. Electrophoretic studies of fragments from defined types of GBG suggested that GBG cleavage induced by complement or properdin activation in serum occurred through this C moiety, since two variants were detectable in one fragment and two were found in the other fragment.

On comparison of fetal-maternal Gb types, approximately one-half the pairs

<sup>2</sup> Goodkofsky, I., I. H. Lepow, and C. A. Alper. Unpublished observations.

<sup>3</sup> Goodkofsky, I., I. H. Lepow, C. A. Alper, and F. S. Rosen. Unpublished observations.

showed differences. This indicated that GBG did not cross the placental barrier.

We thank Dr. Park Gerald for his invaluable suggestions during the course of this work. We are indebted to the members of the Gab, Hin, and Con families for their generous cooperation in providing blood samples.

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