

HEAVY CHAIN SUBCLASSES OF HUMAN γ G-GLOBULIN

SERUM DISTRIBUTION AND CELLULAR LOCALIZATION*

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Investigation of the complex heterogeneity of immunoglobulins in man has led to the finding of subclasses of γ G-globulin, distinguished by partial differences in the heavy or γ -chain. Initially, the subclassification was entirely based on antigenic differences detectable with primate antisera. Thus, Dray (1) described three subclasses of normal human γ G-globulin termed γ_{2a} , γ_{2b} , and γ_{2c} . Variations of these three subclasses in disease were studied by Peetom and Kramer (2). Terry and Fahey (3) have extensively studied normal and myeloma γ G-globulins and have been able to classify most normal and pathological molecules into one of four such subcategories (γ_{2a} , γ_{2b} , γ_{2c} , γ_{2d}). Grey and Kunkel (4), using both rabbit and monkey antisera prepared against individual myeloma globulins, have divided γ G-myeloma globulins and normal γ G-globulins into four subgroups based on heavy chain differences (Vi, Ge, Ne, and a major group comprising 60–70% of all γ G myeloma globulins, We). The apparent relationship among these subclassifications is: $\gamma_{2a} = \text{Ne}$; $\gamma_{2b} = \text{We}$; $\gamma_{2c} = \text{Vi}$; $\gamma_{2d} = \text{Ge}$ (5).

In a previous report (6), we described a similar, less extensive, heavy chain subclassification of γ G-globulin based on differences detected by rabbit antisera prepared against proteins from patients with "heavy chain disease" (7–9). The subpopulations which we have termed Cr, Zu, and Cr variant, are not strictly coextensive with those of other investigators. However, based on

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cross-typing¹ and correlative evidence (4, 5) the Zu subclass is most certainly the same as γ_{2c} and Vi. The Cr subclass contains the majority of normal and pathological γ G-globulins and would appear to be roughly coextensive with γ_{2b} and We. The Cr variant subclass is less well defined. The present paper is concerned with the distribution of these antigenic markers in normal and pathological γ G-globulins, and the biological functions and cellular origins of the γ G-globulin subpopulations.

Materials and Methods

Proteins.—

Franklin proteins: The urinary proteins (Cr and Zu) of two patients with the disorder termed "heavy chain disease" were purified by ammonium sulfate precipitation, ion exchange chromatography, and gel filtration (G-100 Sephadex).

γ G-globulin: Two preparations of normal γ G-globulin were used. One was obtained as Cohn fraction II (Merck Sharp and Dohme, lot 91179). This was purified by chromatography using diethylaminoethyl cellulose equilibrated with 0.01 M, pH 7.5 sodium phosphate buffer. Protein which eluted with the wash buffer front was pooled and pressure-dialyzed to a concentration of 60 mg/ml. As judged by immunoelectrophoresis at this concentration against anti-human serum, no protein other than γ G-globulin was present.

The other γ G-globulin preparation was obtained from a single normal individual (G. B.) by plasmapheresis. Purification was carried out by the Rivanol method (10) followed by ammonium sulfate precipitation, and finally by the chromatographic procedure outlined. This preparation, after pressure dialysis to 60 mg/ml, was also free of other contaminating protein as judged by immunoelectrophoresis.

Myeloma globulins: Some myeloma globulins were prepared by ammonium sulfate precipitation and some by ammonium sulfate precipitation and chromatography. In some instances, samples of whole serum were tested by immunoelectrophoresis and agar double diffusion.

Bence Jones proteins: These were prepared by previously described methods of ammonium sulfate precipitation, DEAE Sephadex (A-50) chromatography, and gel filtration (11).

Antisera.—Male New Zealand rabbits were immunized with a total of 0.5–1.0 mg of purified antigen in complete Freund's adjuvant divided over multiple intradermal sites. After 4 wk, when the antibody levels were sufficiently high, the rabbits were repeatedly bled. Antisera were prepared in this fashion to the Cr protein, the Zu protein, the F_c and F_{a,b} fragments of γ G-myeloma globulins, and to Bence Jones proteins of each antigenic type.

Analytical Techniques.—Immunoelectrophoresis and agar double diffusion were done according to the methods of Scheidegger (12) and Ouchterlony (13), respectively. Ultracentrifugation was carried out in the Spinco model E at 59,780 rpm at 20°C and the values corrected for solvent.

Papain Cleavage.—Enzymatic cleavage using crystalline papain was performed according to the method of Porter (14) as modified by Putnam et al. (15). Hydrolysis was performed at 37°C for 16 hr except where noted. One preparation of γ G-globulin was split using the water-insoluble papain cleavage method of Cebra et al. (16). Hydrolysis of 100 mg protein in 10 ml

¹Dr. W. D. Terry has typed a number of our myeloma globulins according to his system, and it seems well established that the Zu determinant is antigenically the same as γ_{2c} in his classification. Terry et al. (5) have stated that the antigenic determinants of the Cr protein itself have not been clearly identified with monkey antisera. Although the Cr protein appears to be of type γ_{2d} (5), one of our myeloma globulins (Ag) of the Cr type was classed by Dr. Terry as a type γ_{2b} .

of buffer was performed at 37°C for 30 min; the sample was then reduced in 0.005 M cysteine at 37°C for 3 hr. Under these conditions approximately one-half of the sample was reduced to 3.5S fragments. The products were twice passed through a Sephadex G-100 column, and the resultant preparation contained no 7S material in the ultracentrifuge and no immunologically detectable intact γ G-globulin molecules.

Estimation of Relative Concentrations of Subtypes of γ G-Globulins.—The two pools of γ G-globulin (purified Cohn fraction II and purified G. B. preparation) were trace-labeled according to the method of Talmage et al. (17) with ^{125}I obtained as carrier-free sodium iodide from Nuclear Science & Engineering Corporation, Pittsburg, Pa. Uncoupled radioactivity was removed by passage of the sample through Sephadex G-50 columns. The radioactivity in the protein fraction was almost entirely precipitable with 10% trichloroacetic acid (99.2 and 99.6% in the two preparations).

Precipitin curves were constructed using the ^{125}I -labeled γ G-globulin antigen and three rabbit antisera: (a) anti-Cr (unabsorbed), (b) anti-Cr (absorbed with the Zu protein), and (c) anti-Zu (absorbed with the Cr protein). Increasing amounts of antigen were added to 0.5 ml of antiserum and the final volume was adjusted to 1.2 ml per tube. After incubation at 37°C for 1 hr, the tubes were kept at 0°C for 24 hr, then centrifuged at 10,000 rpm. The supernatant was collected, and the sediment was washed three times in chilled 0.15 M sodium chloride.

Aliquots (0.3 ml) of the original supernatants were dissolved in 1 ml Hyamine, and 1 ml Hyamine was added to the washed precipitates. Scintillation fluid was prepared according to the method of Kinard (18). Each sample was added to 5 ml of scintillation fluid and counted in a Packard Tri-Carb scintillation counter. Values of the supernatant aliquots were corrected to full volume. Tubes of control samples of antigen and 0.15 M sodium chloride were treated in the same manner as the precipitates; the radioactivity of these samples was not significantly greater than background.

Fluorescent Antibody Preparation and Staining.—The fluorescent antibody technique of Coons et al. (19) was used to stain splenic cells for the presence of specific antigenic determinants. The methods of Wood et al. (20) and Cebra and Goldstein (21) were used for preparation and purification of immune globulin conjugates. Fluorescein isothiocyanate or tetramethyl rhodamine isothiocyanate (Baltimore Biological Company, Baltimore, Md.) was coupled to the immune globulin preparation of the appropriate antiserum. Splenic tissue prints were obtained from human subjects at postmortem, stained with the various fluorescent conjugates, and examined by ultraviolet light microscopy. The optical filter system described by Cebra and Goldstein (21) was used to objectively discriminate the colors of stained cells.

To prepare the specific fluorescent conjugates, the following procedures were performed in sequence:

1. Ammonium sulfate precipitation (1.6 M) of the appropriate rabbit antisera to obtain the immune globulin fractions.
2. Chromatography using DEAE-cellulose (0.01 M phosphate buffer, pH 7.5).
3. Pressure dialysis to concentrate the unretarded fraction.
4. Absorption with water-insoluble antigen, e.g., an insoluble γ G-myeloma globulin of the Cr type was used to absorb the anti-Zu immune globulin. [The preparation of insoluble antigen by coupling with bisdiazotized benzidine has been described by Bernier and Cebra (22)]. Because of the high degree of solubility of the Zu protein, it was necessary to mix this with equal amounts of inert rabbit γ G-globulin before it could be rendered insoluble by the bisdiazotized benzidine.
5. Conjugation of the absorbed immune globulin preparation to fluorescent dye.
6. When rhodamine was used as the fluorescent dye, the preparation was passed through Sephadex G-50 to remove the excess dye.
7. Fractionation of the conjugated immune globulin by DEAE-cellulose chromatography.

8. Pressure dialysis.

9. The specific fluorescent immune globulin preparations were then tested in preliminary experiments to ascertain optimum protein concentrations and dye to protein ratios. The concentrations employed are given in Table I. Staining by each conjugate could be specifically blocked with the prior addition of a tenfold concentration of nonlabeled immune globulin. Rhodamine-conjugated anti-Zu (absorbed) and fluorescein-conjugated anti-Cr (absorbed) were then mixed prior to application to the splenic prints.

The use of insoluble antigens for absorption in step 4 was necessary to avoid the presence of soluble complexes in the absorbed immune globulin which could produce either nonspecific staining or interference with the other fluorescent conjugate.

TABLE I
Protein Concentrations and Optical Density Ratios of Fluorescent Conjugates Employed in Tissue-Staining Experiments

Preparation	Protein concentration	Optical density ratio	
		$\frac{280m\mu}{495m\mu}$	$\frac{280m\mu}{550m\mu}$
Fluorescein isothiocyanate- anti-Cr-absorbed (green anti-Cr-abs.)	mg/ml 0.8	2.6	
Tetramethyl rhodamine isothiocyanate-anti-Zu- absorbed. (red anti-Zu-abs.)	0.3		5.7

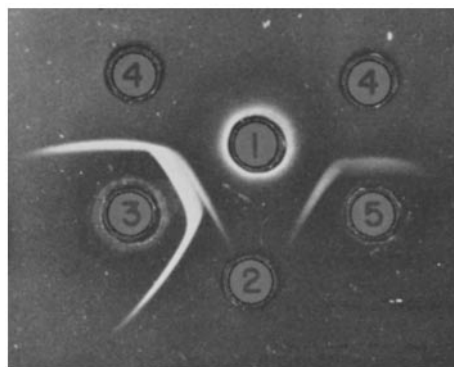
RESULTS

Antigenic Characteristics of the Cr and Zu Proteins.—Antiserum prepared against the Cr protein (anti-Cr) gave reactions of apparent antigenic identity between the Cr protein and either γ G-globulin or the F_c fragment of γ G-globulin. The Zu protein reacted with anti-Cr but was antigenically deficient to the Cr protein. Conversely, antiserum to the Zu protein (anti-Zu) showed the Zu protein to possess antigenic determinants undetected in the Cr protein. Moreover, the anti-Zu antiserum produced two precipitin lines with γ G-globulin. In Text-fig. 1 the line closest to the antiserum well gave a reaction of partial identity with the Zu protein, and the line closest to the center well was antigenically identical with the Zu protein. In immunoelectrophoresis both lines were in the γ -globulin region. These studies indicate: (a) the Cr and Zu proteins share some antigenic determinant; (b) each has a "specific" determinant not shared by the other; and (c) both "specific" determinants are detectable in normal γ G-globulins.

Antisera to the "specific" determinants of the Cr and Zu proteins were prepared by absorption with the heterologous antigen (e.g., anti-Cr after absorp-

tion with the Zu protein was "specific" for the Cr determinant). With anti-Cr-absorbed and anti-Zu-absorbed antisera it was possible to demonstrate that one population of normal γ G-globulin molecules possesses the Cr determinant, and another population possesses the Zu determinant.

Cr and Zu Types of Normal γ G-globulins.—The "specific" Cr and Zu antigenic determinants were studied with regard to their frequency in the general population, the percentage of normal γ G-globulin molecules bearing the "specific" determinants, and the location of the specific determinants on the γ G-globulin molecule.

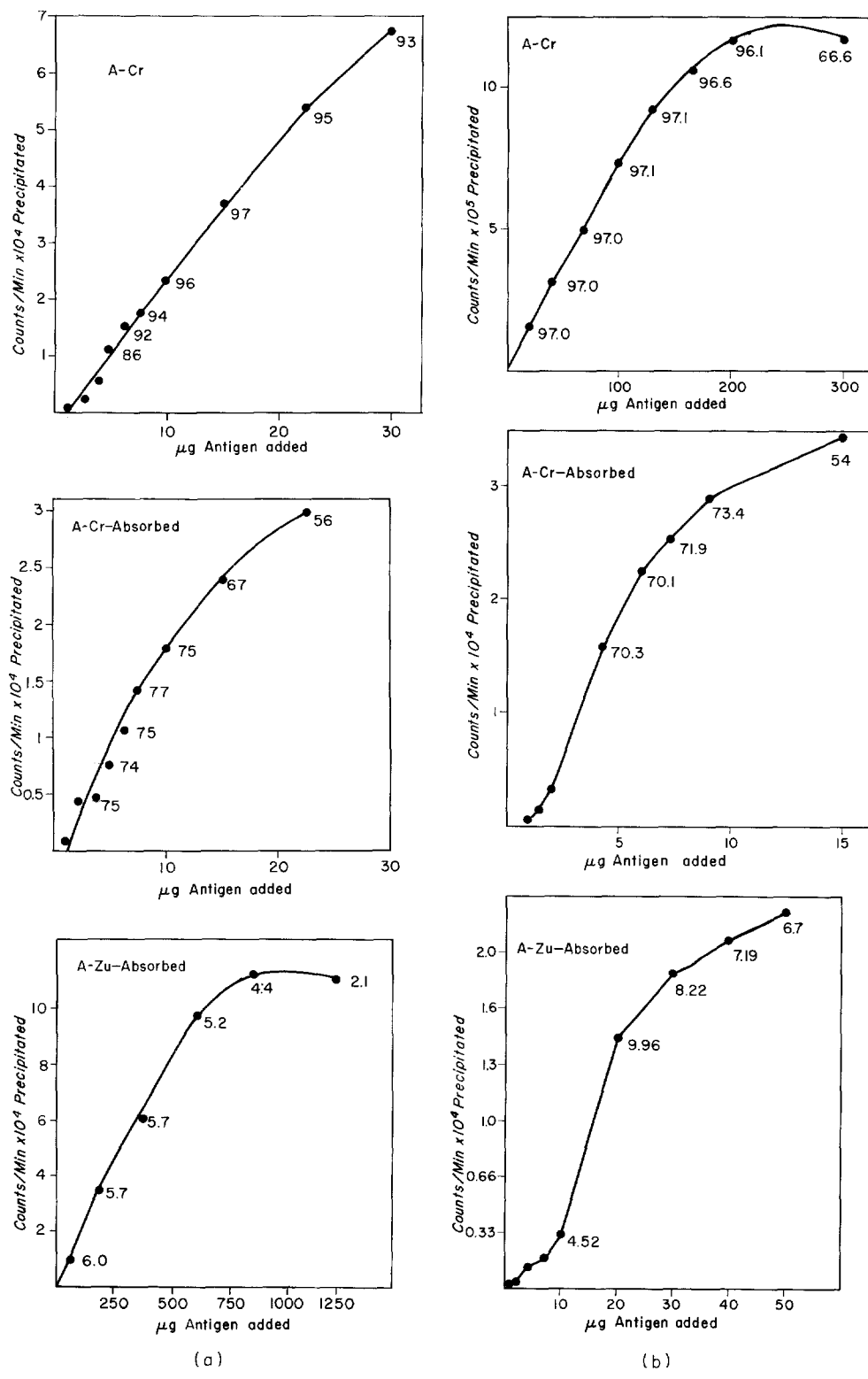


TEXT-FIG. 1. Ouchterlony double diffusion showing reactions with anti-Zu (well 3) and anti-Zu (absorbed with the Cr protein) (well 5). Antigen wells contain normal γ G-globulin (well 1) the Cr protein (well 2), and the Zu protein (well 4). A double line develops between anti-Zu and normal γ G-globulin. The line closest to the anti-Zu fuses with the Cr protein, and the other line fuses with the Zu protein. The anti-Zu absorbed with the Cr protein (well 5) still reacts with normal γ G-globulin and with the Zu protein.

Normal sera: The sera of 250 normal blood donors were tested with anti-Cr and anti-Zu antisera, and each normal serum was found to possess "specific" Zu and Cr determinants. A number of sera of known Gm specificity [Gm (a+ b-), Gm(a- b+), Gm (x)]² were tested, and each serum was found to possess both determinants. In each instance a single line was formed with unabsorbed anti-Cr while two lines were formed with unabsorbed anti-Zu.

Relative percentages: The percentage of γ G-globulin molecules possessing the Cr determinant and the percentage possessing the Zu determinant were determined by quantitative precipitin curves using ¹²⁵I-labeled γ G-globulin from a single individual (Text-fig. 2 a). Anti-Cr before absorption precipitated approximately 97% of the radioactivity; after absorption with the Zu protein, it precipitated approximately 75%. Anti-Zu, on the other hand, after absorp-

²Sera of known Gm specificities were given to us by Dr. Wallace Epstein and Dr. A. G. Steinberg.



TEXT FIG. 2

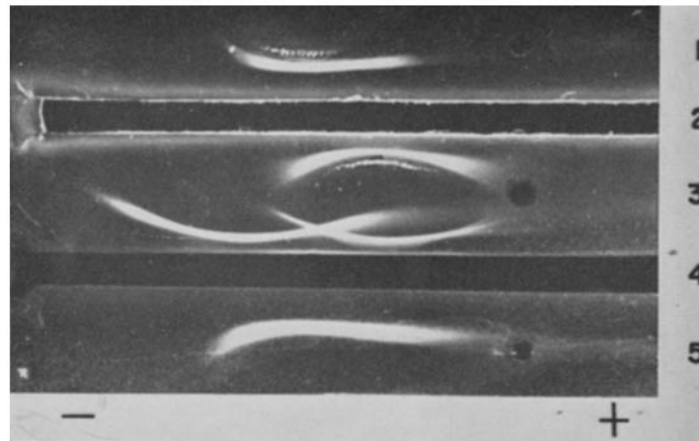
tion with the Cr protein, precipitated only 5-6% of the radioactive antigen. Similar, though not identical, results were obtained when a pool of γ G-globulin (Cohn fraction II) was used as the radioactive antigen (Text-fig. 2 *b*). Thus, when corrected for the 3% of radioactivity not precipitated by the unabsorbed anti-Cr, the individual preparation contained 77% Cr-type molecules, 6% Zu-type molecules, and (by difference) 17% molecules lacking both determinants. Similarly, the pooled γ G-globulin preparation contained 74% Cr-type molecules, and 10% Zu-type molecules. By difference, 16% of molecules lack both determinants.

Molecular location: After papain digestion of γ G-globulin under the conditions of Porter (14), F_c and F_{ab} fragments are obtained. The Cr determinant was found by immunoelectrophoresis in the F_c fragment of normal γ G-globulin, and in the F_c fragments of several myeloma globulins. However, the Zu determinant could not be detected in either the F_c or F_{ab} fragments of γ G-globulin digested in this fashion. When controlled hydrolysis with water-insoluble papain was performed [according to the method of Cebra et al. (16)], the Zu determinant was found in the F_c fragment (Text-fig. 3). The lability of the Zu determinant to lengthy papain hydrolysis suggests that its location in the polypeptide chain is extremely close to the point of papain cleavage. By inference, the Cr determinant would be in an analogous position but might be relatively more resistant to papain degradation. It should be pointed out that Takatsuki and Osserman have described differences in products of papain hydrolysis of Zu and Cr proteins (23).

Classification of γ G-Myeloma Globulins.—Since individual myeloma globulins appear to be single homogeneous molecular species of γ -globulin, the incidence frequency of specific antigenic characteristics in a large series of myeloma globulins would be expected to approximate the frequency of that characteristic in a pool of normal γ -globulin molecules. This has been shown to be the case with the relative incidence of types K and L. Type K molecules are approximately twice as common as type L molecules in normal pools and in a large series of myeloma globulins (24, 25). Accordingly, 108 γ G-myeloma globulin specimens were tested for the presence of the Cr and Zu determinants with antisera rendered specific for these determinants by absorption. The

TEXT-FIG. 2. Precipitin curves using ^{125}I -labeled γ G-globulin obtained from (a) a single individual (G. B.) and (b) purified Cohn fraction II, normal human pool. The symbol in the upper left corner designates the antiserum used in the construction of the precipitin curves. The numerical values given at points along the curves indicate the percentage of radioactive antigen precipitated by the antiserum. The unabsorbed anti-Cr antiserum (A-Cr) at the maximum precipitated 97% of the γ G-globulin in each case. The anti-Cr antiserum absorbed with the Zu protein (A-Cr absorbed) precipitated 73 and 77% of the radioactive antigen maximally in the two instances. The anti-Zu antiserum, absorbed with the Cr protein (A-Zu absorbed) precipitated 10 and 6% of the radioactive antigen maximally.

findings are shown in Table II. The relative incidence of Zu-type γ G-myeloma globulins (7.4%) and of the Cr-type (81.5%) is remarkably similar to the distribution of these molecules in the normal γ G-globulin pool and in the serum of the one individual studied. An additional number of γ G-myeloma globulins either lacked the Cr and Zu determinants entirely (0.9%), or they possessed some of the Cr determinants but were antigenically deficient to the Cr protein and the other Cr-type myeloma globulins (10.2%). Those with some but not all of the Cr determinants are called Cr variants.



TEXT-FIG. 3. Immunoelectrophoresis of normal γ G-globulin (1 and 5) and of normal γ G cleaved with water-insoluble papain (3). The antisera are anti-Zu (2) and anti- γ G-globulin (4). Anode is at the right. The anti- γ G-globulin reacts with the intact γ G-globulin (5) and with Fc and Fab fragments (3). The anti-Zu reacts with the intact γ G-globulin (1) and with the Fc fragment (3). The inner, "specific Zu" line is found in both reactions. (The inner line has been accentuated in this figure for clarity.)

Most of the myeloma globulins were studied in terms of light chain type as well as heavy chain subgroup. As is shown in Table II, the K:L ratio of the entire group of myeloma globulins and of the Cr-type subgroup was slightly higher than the expected 2:1 ratio. In the relatively small number sampled, the K:L ratio of the Cr variants was near unity and conversely, the K:L ratio of the Zu-type globulins was higher than would be expected. However, these differences probably reflect sampling. For instance, in terms of the Zu group, Grey and Kunkel found eight type K and two type L light chain types in 10 Vi group myeloma proteins (4) and Terry, Fahey, and Steinberg found 13 type K and 14 type L light chain types in 27 γ_{2c} -myeloma globulins (5). Hence, in the cumulative number of 45 myeloma globulins in the three series typed as Zu, Vi, or γ_{2c} , 28 are type K and 17 are type L. These values are reasonably close to the 2:1 ratio randomly expected.

Of the 11 proteins classified as Cr variants, eight were studied as regards electrophoretic mobility in agar gel electrophoresis and all eight were found to have mobilities in the slow β - or fast γ -globulin range. This was in marked contrast to the Cr and Zu groups where the vast majority of myeloma proteins had a γ -globulin mobility. The one myeloma globulin (Ca) which could not be classified also had a β -globulin mobility. These findings are of interest in terms of the Ge subgroup of Grey and Kunkel which is also comprised of proteins with rapid electrophoretic mobility (4).

Biological Considerations.—The Cr and Zu types of normal γ G-globulin were studied regarding antibody function and other biological characteristics. The presence of the two antigenically defined types of γ G-globulin was deter-

TABLE II
Classification of γ G-Myeloma Globulins Based on Heavy Chain Antigenic Determinants, and the Light Chain Class Distribution within These Subgroups

Heavy chain classification			Light chain classification		
Type	Number	Per cent	Number typed*	Type K	Type L
Cr type	88	81.5	75	52	23
Cr variant	11	10.2	11	6	5
Zu type	8	7.4	8	7	1
Not classified	1	0.9	1	1	0
Total	108	100	95	66	29

* Light chain typing was not performed on 13 Cr-type γ G-myeloma globulins.

mined in: (a) a specific immune precipitate, (b) fetal cord blood, (c) neonatal sera, and (d) severe hypogammaglobulinemia.

(a) Serum was obtained from a single individual immunized with diphtheria toxoid. It was reacted with diphtheria toxoid, and the immune precipitate was centrifuged, washed, dissolved in acid, and separated from the antigen by passage through Sephadex G-200. When this specific antibody was tested in immunodiffusion, both the Cr and Zu populations were present. In this experiment, there appeared to be a relative increase in concentration of the Zu molecules acting as antibodies, but both types of γ G appeared to be effective.

(b) Placental transfer of the two types of γ G-globulin was determined by examination of six paired samples of maternal serum and fetal cord blood. In each instance, both Zu and Cr populations were present, with a predominance of the Cr type. Since γ G-globulin synthesis in the fetus is negligible (26), these results appear to indicate that placental transfer of both types of γ G-globulin occurs.

(c) Sera from 16 infants varying in age from 7 wk to 24 months were

studied by immunodiffusion with use of unabsorbed antiserum. In all cases both types of γ G-globulins could be detected. The relative amounts of the two populations seemed to be comparable to the ratio found in the serum of normal adults, as judged from the position of the precipitin lines in the immunodiffusion plates. This was the case even in infants of 7 and 10 wk of age. Both classes of molecules appeared antigenically identical with those seen in adult sera.

(d) Sera from three adult patients with severe hypogammaglobulinemia (30, 100, and 100 mg of γ G-globulin per 100 ml of serum, respectively) were examined by immunodiffusion. These sera were chosen because the patients had *not* received therapeutic injections of γ -globulin. The two sera containing 100 mg/ml could readily be shown to have both populations. When relatively large amounts of the more deficient serum were used as antigen, both the Cr and Zu populations of γ G-globulin were detectable. Again, the same relative proportion of molecules appeared to be present as in sera from normal patients, indicating that the virtual agenesis of γ G-globulin in these individuals was not selective in terms of either the Cr or Zu populations.

Thus, in the four biological situations—antibody activity, placental transfer, neonatal synthesis, and severe hypogammaglobulinemia—the two antigenically distinguishable populations of γ G-globulin exhibited the characteristics of classical γ G-globulin antibodies, and the relative proportion of the two populations was approximately the same as that of normal serum. Since precise quantitation was not performed, moderate variations may, of course, be present.

Cellular Localization.—The cellular sites of synthesis of Cr and Zu determinants were investigated by use of fluorescent antibody techniques. Insoluble antigens were used to absorb the antisera before conjugation with fluorescent dyes in order to leave the antisera free of any excess antigen as well as of cross-reacting antibodies. Human splenic prints were stained with the two fluorescent conjugated antisera, both sequentially and in mixtures. Fig. 1 and Table III show the results of staining splenic tissue prints with mixtures of red anti-Zu-abs. and green anti-Cr-abs. Cells stained either red or green, but not at all with both reagents. The percentage of cells stained with green anti-Cr-abs. was considerably greater than the percentage stained with red anti-Zu-abs. This 9:1 ratio approximates the ratio of the serum concentration of the two γ G-globulin types, and also the relative incidence of the two types of myeloma globulins.

As a control, fluorescent antisera directed against the two types of light chains (κ , λ) were used in combination with either the red anti-Zu-abs. or the green anti-Cr-abs. Results of one experiment are presented in Table III. Previous experiments with this particular spleen (specimen 6 of reference 22) had shown that 43% of the immunoglobulin-containing cells contained γ G-globulin, and that 44% of the immunoglobulin-containing cells contained type L light

chains. When this specimen was stained with red anti-Zu abs. and green anti-type L light chain, 6% of cells stained red, 4% were mixed, and 90% were green. The green and mixed cells are all those containing immunoglobulins of type L (total, 94%), but only 43% of these contain γ G-globulins. Therefore, of 100 cells staining in this experiment, 41 may be considered to contain type L γ G-globulin. Four of these (the mixed cells) also contain Zu-type heavy chain (9.8%). Hence, the Zu-type heavy chain-containing cells comprise about one-tenth of the γ G-globulin-containing cells.

The ratio of red cells to mixed cells (3:2) is the ratio expected assuming that the red cells contain a type κ -light chain (22).

TABLE III
Percentages of Stained Cells in Splenic Tissue Preparations Showing Red, Green, or Mixed Fluorescence

Reagents	Spleen No.	Red	Green	Mixed
Red anti-Zu-abs. and green anti-Cr-abs.	I	6	94	0
	II	14	86	0
	III	9	91	0
Red anti-Zu-abs. and green anti- λ	II	6	90	4

DISCUSSION

The two antigenically distinguishable subclasses of γ G-globulins studied appear to have extensive similarities to one another in terms of biological characteristics, chemical structure, and antigenic features. The studies reported here are, however, more concerned with the differences which can be shown to exist.

Numerically, the Cr type of normal γ G-globulin molecules are approximately 10 times more common than the Zu. Similarly, myeloma globulins possess the Zu determinant at one-tenth the frequency of the Cr determinant. As judged by the myeloma analogues of these populations in the peptide-mapping studies of Frangione and Franklin (27) and of our laboratory,³ definite differences in primary sequence occur and account in part for the observed antigenic differences between the two populations. From the studies cited, it would appear that the area where sequence differences occur is in that portion of the molecule which is susceptible to the prolonged action of papain. It is pertinent in this regard that Grey and Kunkel (4) have found that the antigenic characteristics of the Vi class of proteins are destroyed, at least partially, by papain, yet are preserved when proteolysis with pepsin is performed.

³Brackenridge, C., F. W. Putnam, and C. W. Easley. Comparative structural study of the heavy chains and Fc fragments of normal and pathologic G immunoglobulins. In preparation.

It has been demonstrated by the fluorescent antibody techniques described that individual cells do not contain both types of γ G-globulin heavy chains. We may infer that this means a given γ G-globulin-producing cell does not synthesize both the Zu or Cr type of heavy chain, at least at one time in the cell's existence. This may indicate a degree of cellular predifferentiation and is in accord with the findings of Bernier and Cebra (22) regarding the cellular localization of the major classes of immunoglobulin polypeptide chains and with the close correlation between numbers of cells containing individual chains and the relative serum concentration of the chains. These findings accord with many of the observations of Chiappino and Pernis (28, 29). However, these authors did find cells staining for more than one kind of light chain or heavy chain in the germinal center of lymph nodes.

Finally, the complex heterogeneity of the immunoglobulins has been increasingly recognized in recent years. In addition to the class and type categories, subcategories have been demonstrated for the γ G-globulins and, as has been shown by Stein et al. (30), for Bence Jones proteins. Similar subcategories have now been found for the γ A- and γ M-globulin classes (31-33). Added to these subcategories are the individual segmental differences in amino acid sequence which presumably confer antibody specificity to an individual molecule. Attempts are underway in various laboratories to delineate these latter differences by analogy in the case of Bence Jones proteins (34-36). When the amino acid sequences of a number of related Bence Jones proteins are completed, it is to be expected that a formulation of the biosynthetic mechanisms of antibody formation will be more feasible.

In spite of the apparent, endless chemical complexities revealed by detailed studies of the immunoglobulins, it seems clear that the many facets of immunoglobulin heterogeneity will have to be appreciated and explained before a unified hypothesis can provide an understanding of these molecules as products of protein synthesis.

SUMMARY

Two antigenic types of naturally occurring proteins related to the papain produced F_c fragment of γ G-globulin have been studied. Most normal and myeloma γ G-globulins are related to one or the other of these proteins, based on antigenic and structural characteristics of their heavy chain. Molecules bearing the determinant "Cr" are approximately 10 times as abundant as those bearing the determinant termed "Zu" in pools of normal γ G-globulin and among a large group of γ G-myeloma globulins. Both populations have properties of typical γ G-globulin antibodies. Splenic cells, stained for these two populations with specific fluorescent antibody preparations, are found to contain one or the other of the two populations of γ G-globulin, but not both. Approximately 10 times as many cells contain the Cr determinant as contain the Zu determinant.

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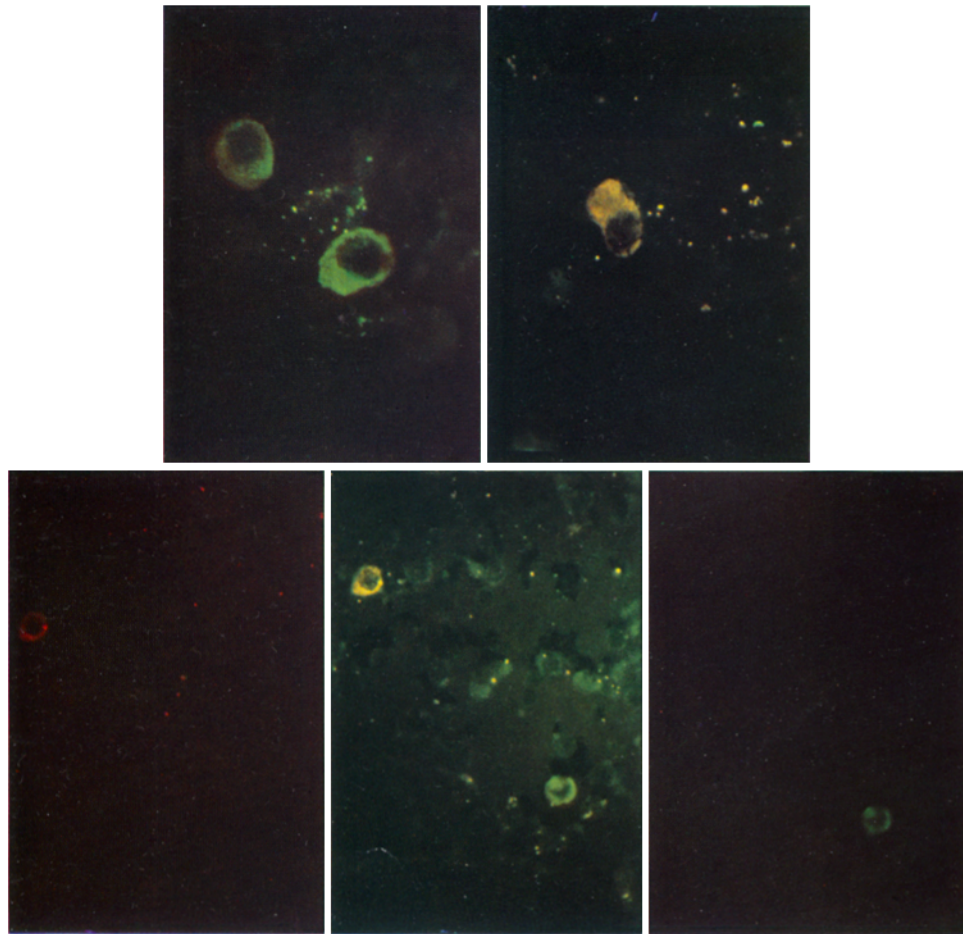
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PLATE 22

FIG. 1. Photomicrographs of human splenic prints stained with a mixture of rhodamine-conjugated anti-Zu (absorbed with Cr) and fluorescein-conjugated anti-Cr (absorbed with Zu). The top row shows two green stained cells (left) and one red stained cell (right). Both fields viewed with a neutral K-2 filter and a 100 \times oil-immersion objective. The bottom row shows three pictures of one field as viewed with the neutral K-2 filter (center), a 57A red-excluding filter (right) and a 23A green-excluding filter (left). A green-staining cell and a red-staining cell are evident in the center picture. The green fluorescence passes the 57A filter, and the red fluorescence passes the 23A filter.



(Bernier et al.: γ G-globulin subclasses)