Analytical Review

Human Gamma Globulin

By T. B. Tomasi, Jr.

In 1939, Tiselius and Kabat demonstrated that antibody activity was localized by electrophoresis primarily in the slowest moving, γ-globulin fraction of the serum proteins. Subsequent studies have shown that antibody activity is associated with a group of multichained proteins of varying mobility and molecular size. These proteins have in common the ability to interact more or less specifically with the material eliciting their formation and also certain structural similarities which are responsible for their antigenic cross-reactions. The group of proteins composing the γ-globulins or as they are frequently called, immunoglobulins, include 7S γ, 19S γ, γ1A, and the low-molecular weight γ-globulins found primarily in urine. In addition there is the recently described protein related to the H chain of 7S γ which may represent a naturally occurring subunit of γ-globulin. The terminology recently proposed at the conference on the "Nomenclature for Human Immunoglobulins" held in Prague in May 1964, along with the terms in current usage are outlined in table 1.

Structure of 7S γ-Globulin

An important contribution to the understanding of the structure of antibodies has been the discovery by Edelman that 7S γ-globulin is a multichained molecule and that the chains are cross-linked by disulfide bridges. The 7S γ-globulin molecule consists of two types of polypeptide chains: L (light) or B chains of molecular weight approximately 20,000 and H (heavy) or A chains of molecular weight approximately 60,000. These two types of chains can be isolated by treatment of the 7S molecule with disulfide bond reducing agents followed by alkylation and subsequent chromatography on Sephadex or ion-exchange cellulose columns. The conditions of chromatography must include agents such as urea, acid, or detergents which prevent association of the chains through noncovalent bonds. The participation of noncovalent forces is evidenced by the fact that treatment of 7S γ-globulin by reducing agents alone does not result in dissociation or loss of antibody activity. In addition, under appropriate conditions, isolated L and H chains in which the SH groups have been blocked by alkylation will interact to reform 7S molecules.

According to amino acid analysis, there are approximately 14 disulfide bonds in human 7S γ-globulin. It is known that different disulfide bonds vary in the ease with which they can be reduced; those which are most difficult to reduce are probably intrachain disulfides some of which may be buried in the interior of the molecule. Several of the more labile interchain disulfides includes those linking the L and H chains to each other and the critical bond

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Rowe & Sahey (J. Exper. Med. 121:171, 1965) have recently described a new immunoglobulin class, IgD, which has a median level of .03 mg. per ml. in normal serum.
Table 1.—Nomenclature for the Human Immunoglobulins

<table>
<thead>
<tr>
<th>Present Terms</th>
<th>New Terms</th>
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<tbody>
<tr>
<td>7Sγ, γG, γM</td>
<td>γ G or IgG</td>
</tr>
<tr>
<td>γ11, B2A</td>
<td>γ A or IgA</td>
</tr>
<tr>
<td>19Sγ, γ1M, B3M</td>
<td>γ M or IgM</td>
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<tr>
<td>Type I or B proteins</td>
<td>K</td>
</tr>
<tr>
<td>Type II or A proteins</td>
<td>L</td>
</tr>
<tr>
<td>Type I or B L chains</td>
<td>k (kappa)</td>
</tr>
<tr>
<td>Type II or A L chains</td>
<td>λ (lambda)</td>
</tr>
<tr>
<td>Heavy chains 7Sγ</td>
<td>γ (gamma)</td>
</tr>
<tr>
<td>Heavy chains γ1A</td>
<td>α (alpha)</td>
</tr>
<tr>
<td>Heavy chains γ1M</td>
<td>μ (mu)</td>
</tr>
<tr>
<td>Fragment A, C, S, I, II</td>
<td>Fab-fragment</td>
</tr>
<tr>
<td>Fragment B, F, III</td>
<td>Fc-fragment</td>
</tr>
<tr>
<td>A piece</td>
<td>Fd-fragment</td>
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linking the two univalent 3.5 S antibody fragments described by Nisonoff et al.12 Palmer et al.13 have shown that reduction of rabbit γ-globulin followed by acidification results in the formation of large amounts of half molecules. The half molecules are presumably linked to each other through their H chains by a single disulfide as depicted in figure 1. Thus only three or four of the disulfide bonds present in the native molecule have been even partially localized. The remaining bonds are either intrachain or possibly link subunits of the H chains. The latter possibility has been suggested by the recent report of Franklin et al.14 of a patient with the so-called "heavy-chain" disease. The abnormal protein in this disease appears to be closely related to the F fragment of 7S γ. If this is indeed a true structural unit of the 7S molecule, then the H chain may consist of two subunits and 7S γ-globulin of six polypeptide chains. However, no direct evidence is presently available for this thesis and it has not been possible to obtain further reduction of isolated H chains.

Porter15,16 first made the important observation that treatment of γ-globulin with papain in the presence of cysteine results in dissociation of the 7S molecule to 3.5 S fragments. The papain digest can be separated by electrophoresis into two fractions—S (slow) and F (fast)17 and by chromatography on carboxymethylcellulose into three fractions—I, II, and III of Porter15 and A, B, C of Franklin.15 Fractions I and II (A and C of Franklin) possess univalent antibody activity and each fraction has been shown to be derived from different populations of 7S γ-globulin molecules;19 both univalent fragments of a given antibody appearing in the same chromatographic fraction.20 Fraction III (Franklin's B) or F fragment is devoid of antibody activity but contains the carbohydrate moiety and the determinants responsible for class specificity. The F fragment is also responsible at least in large part for certain other properties characteristic of the 7S molecule such as: skin fixation,21 placental permeability,22 Gm specificity,21,24 complement fixation,25,27 fixation to microsomes,28 and reaction with rheumatoid factor.29-31

Treatment of 7S γ-globulin with pepsin destroys a large portion of the molecule which closely resembles the F fragment of the papain digest. The residual portion of the molecule has a molecular weight of approximately 105,000 (5S) and retains bivalent antibody activity. Treatment of the 5S fragment with cysteine reduces a single labile disulfide bond resulting in two
Fig. 1.—Schematic model for the 7S antibody molecule proposed by Edelman and Gallo (slightly modified) illustrating the various types of cleavage of the molecule.
univalent 3.5S fragments which are similar (although perhaps not identical) to those produced by the action of papain.\textsuperscript{12} Presently available evidence derived from sedimentation,\textsuperscript{15} electrophoresis,\textsuperscript{32} chromatography,\textsuperscript{33} and fingerprinting data,\textsuperscript{34} suggests that the two univalent fragments are very similar, if not identical. Evidence for the univalency of the fragments is provided by hapten binding studies,\textsuperscript{35,36} inhibition of the homologous precipitin reaction,\textsuperscript{15} and the binding to cells of fragments derived from anti-Rh and other red cell antibodies.\textsuperscript{37,38} The fragments derived from red cell antibodies are truly univalent, since unlike “incomplete” unfragmented 7S antibodies, they do not agglutinate cells even in a high protein medium, or after prior treatment of the erythrocytes with trypsin or other proteolytic enzymes.

It has been possible using purified antibody to reoxidize the critical S-S bond and restore precipitating activity.\textsuperscript{39} If univalent fragments derived from antibodies of different specificity are mixed, hybrid molecules are formed apparently as a result of random recombination of the fragments.\textsuperscript{40} These hybrids precipitate or agglutinate only with a mixture of the two antigens in accordance with the prediction of the lattice theory.\textsuperscript{40,41}

Olins and Edelman\textsuperscript{42} have conclusively confirmed the previous suggestions\textsuperscript{42,43} that the 7S \(\gamma\)-globulin molecule consists of two L and two H chains. These workers found that 7S molecules reconstituted from L and H chains, each labeled with different isotopes, contained the isotope in a molar ratio near unity. From this and the molecular weights of the chains it was calculated that the native molecule must contain two L and two H chains. Similar results have been obtained by Marler et al.\textsuperscript{44} for rabbit \(\gamma\)-globulin. The results of these experiments are most consistent with a symmetrical molecule, each half of which consists of an L and an H chain pair, in which the two chains of a pair cooperate to produce an antibody combining site. The recovery of specific antibody activity from the mixing of separate chains\textsuperscript{45,46} and the close resemblance of reconstituted molecules to native 7S \(\gamma\)\textsuperscript{47} support the thesis of chain interaction in the formation of the antibody combining site. Similar conclusions have been reached by Metzger et al.\textsuperscript{48} using the method of affinity labeling. However further work is needed to clarify the exact contribution of each type of chain to the activity of the antibody combining site.

Several types of measurements including hydrodynamic,\textsuperscript{49} electronmicroscopic,\textsuperscript{50} and low angle x-ray scattering studies\textsuperscript{51} indicate a length of approximately 240 \(\AA\) for the 7S antibody molecule. According to the x-ray scattering studies of Kratky et al.\textsuperscript{51} the 7S \(\gamma\)-globulin molecule appears to be a cylinder of elliptical cross section with dimensions 240 \(\AA\) \(\times\) 19 \(\AA\) \(\times\) 57 \(\AA\). The manner in which the chains are arranged with respect to one another as well as the details of the folding of the individual chains has not been elucidated. It seems likely, however, from the recent studies of Almeida et al.\textsuperscript{50} that the antibody combining sites are located at the two ends of the molecule. Optical rotatory dispersion studies of human 7S,\textsuperscript{52} 19S,\textsuperscript{52,53} and \(\gamma_1\),\textsuperscript{54} as well as several animal \(\gamma\)-globulins,\textsuperscript{54} fail to demonstrate evidence of significant amounts of alpha-helical content. All of the various types of \(\gamma\)-globulins as well as the fragments derived from papain\textsuperscript{55} have shown strikingly similar optical properties. The absence of significant helical content may be related to the relatively high
content of those amino acids (valine, isoleucine, serine, threonine, and proline) which according to Blout are poor alpha-helix formers. The relatively large number of disulfide bonds in γ-globulin may also restrict alpha-helix formation. A conformation consistent with the dispersion data recently proposed by Imahori is the cross beta structure. Evidence for this type of structure is derived both from the optical rotatory dispersion measurements and from studies of oriented films of γ-globulin with polarized infrared light. The apparent similarity of the configurations of certain animal γ-globulins to that of human may explain why the polypeptide chains from various species will reassociate with one another to form 7S molecules. A model for the 7S antibody molecule consistent with the chemical and physical studies discussed above has been proposed by Edelman and Gally and is represented in schematic form in figure 1.

STRUCTURE OF γ1M AND γ1A MOLECULES

Much less information is available regarding the details of the structure of the γ1M and γ1A molecules. Both contain L chains carrying the common determinants which are responsible for the cross reactions between the various immunoglobulin classes. Normal γ1M and γ1A, isolated γ1A myeloma proteins, and pathologic macroglobulins all show urea starch gel patterns following reduction and alkylation which are in general quite similar to those of the 7S γ-globulins. The L chain areas of the γ1A myeloma proteins and pathologic macroglobulins show the typical discrete banding characteristic of homogeneous proteins. The H chains of the macroglobulins and γ1A proteins differ somewhat in mobility from that of 7S γ-globulins.

The 19S γ1M globulins dissociate to 7S units on treatment with reducing agents in the absence of urea. It has been estimated that approximately six 7S subunits are present per mole of 19S protein. The 7S subunits generally possess only one electrophoretic component and contain all of the immunologic specificity of the parent molecule. In most reports, 19S agglutinating and precipitating antibody activity disappears completely following reduction, and loss of activity has been commonly used as one criteria that an antibody is of the 19S type. However, in several recently reported experiments, activity has been found in the 7S subunits including those from: Rh agglutinins, isohemagglutinins, and rheumatoid factor. Reaggregation of 7S subunits by removal of the reducing agent results in a return of approximately 90 per cent of the original activity, both of macroglobulins having rheumatoid factor activity and with the isohemagglutinins. Mixtures of subunits derived from 19S anti-A and anti-B antibodies followed by reassociation produced 10-20 per cent of “mixed molecules.” The formation of “mixed molecules” along with the studies showing various activities in the macroglobulin subunits suggests that the intact 19S molecule has a valence greater than 2 and probably in the 6-12 range.

The γ1A of normal serum is primarily (90+ per cent) 7S, although small amounts of γ1A polymers are also present. The γ1A polymers have a variety of sedimentation rates (9-19S) and are commonly found in the sera of pa-
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Like the 19S γ1M proteins, they dissociate on treatment with reducing agents to 7S subunits. Isoagglutinin activity has been found to be associated with both intermediate77 and 19S γ1A polymers.74

One of the most striking differences between 7S and γ1A and γ1M is in their carbohydrate content; 7S γglobulin contains approximately 2.5 per cent carbohydrate, and the γ1A and γ1M globulins approximately 10 per cent. The monosaccharide units of 7S γ-globulin include galactose, mannose, fucose, glucosamine, and sialic acid. It seems likely that in the 7S molecule a single type of carbohydrate side chain is present which is covalently linked to an aspartyl residue of the protein, although the exact number of carbohydrate side chains per molecule has not been determined there appears to be a small number, most likely two, one attached to each H chain. The type of bond linking the carbohydrate unit to the protein is uncertain but likely possibilities include an amide bond between the beta carboxyl group of aspartic acid and the 2-amino group of glucosamine, and an acyl glycosylamine linkage between glucosamine and the amide nitrogen of an asparagine residue. The sequence of the monosaccharides has not been established except that a glucosamine is attached to the protein and the sialic acid occupies a terminal position. It is the negative charge on the terminal sialic acid which is responsible, at least in part, for the faster electrophoretic mobility of the γ1 proteins. Using the enzyme neuraminidase the sialic acid can be removed from the γ1 molecule resulting in significant slowing in mobility.74

**Electrophoretic Heterogeneity**

Various types of zone electrophoresis including starch block, starch gel, and immunoelectrophoresis all demonstrate considerable electrophoretic heterogeneity in the γ-globulins. In immunoelectrophoresis, heterogeneity is especially well shown in the typical long arc of 7S γ-globulin which extends well into the beta region. Recent studies by Kunkel et al. show that highly purified human antibodies against well-defined antigens give a localized arc on immunoelectrophoresis. This suggests that the long arc of 7S γ may be a composite of many localized arcs of individual antibodies. Homogeneity of individual antibodies is also suggested by the work of Edelman et al. showing that dissociated (by reduction and alkylation) guinea pig antibodies against haptenes yield urea starch gel patterns which have a characteristic number and mobility of the fast bands corresponding to the L chains. The slower of the two areas on the starch gel patterns represents the H chains. The H chain bands of different 7S antibodies are quite similar and techniques other than electrophoresis (antigen analysis) are required to show H chain heterogeneity.

Studies on isolated human antibodies against well-defined antigens (dextran, levans, blood group A substance, tetanus toxoid) also show characteristic banding patterns which differ for antibodies of different specificity. It appears that, in general, the more restricted the antigenic determinant, the simpler the L chain pattern. In one case presented there was a single prominent L chain band. This is the type of pattern which would be expected for...
a homogenous antibody made against a single determinant in which the antibody contains two identical L chains.

Although both isolated antibodies and purified myeloma proteins show relatively simple L chain patterns, they most frequently show multiple bands. Of the several possible explanations for multiple banding, it seems likely that in many cases it is related to the heterogeneity of antibodies which results from slight differences in specificity. This has been shown to be the case with guinea pig antibodies to DNP (dinitrophenyl haptene). The increasing complexity of L chain patterns which results as immunization proceeds is consistent with the appearance of antibodies of slightly different specificities as larger varieties of cell types are recruited.

**Heterogeneity in Molecular Size**

Although high molecular weight γ-globulins were described by Pederson some 20 years ago, it is only recently that their significance as a distinct class has been appreciated largely through the work of Kunkel on high molecular weight antibodies and Waldenström on the pathologic macroglobulins.

Waldenström first described a group of patients characterized by the presence of large amounts of highly homogeneous 19S proteins in their sera. It seems likely that these, like the myeloma proteins, are the products of a single clone of cells and represent high molecular weight antibodies whose antigens are not yet known. In a few cases, however, antibody-like activity (cold-agglutinins, sheep cell agglutinins, rheumatoid factor, and autoimmune complement-fixing activity) has been demonstrated in these sera.

Kunkel has reviewed the chemical and immunologic properties of 19S antibodies in detail. These include a number of classical antibodies such as horse antipneumococcal, human antityphoid, and isoagglutinins, as well as a group of less well-defined proteins usually classified as antibodies i.e., leukagglutinins, cold agglutinins, conglutinins, heterophile, and Wasserman antibodies. Although the 19S antibodies are commonly directed against complex antigens such as bacteria and cells, they are also elicited by simple globular proteins. The 19S antibodies are formed mainly in the early stages of primary immunization and are followed by antibodies of the 7S variety. Secondary immunization elicits primarily 7S antibodies. Apparently the 19S response has little, if any, immunologic memory and requires the persistence of antigen for continued synthesis. Similarly in the neonatal period antibody is primarily 19S until about the third month when a rapid change to the 7S type occurs. Passive administration of 7S antibody inhibits the subsequent antibody response, whereas administration of 19S antibody in equal or higher titers does not. The possibility of feedback control involving 7S antibody is also suggested by recent studies showing that in rabbits treated with small doses of 6-mercaptopurine only 19S antibodies were formed and the serum titers were markedly and rapidly suppressed by passively administered homologous 7S antibody. It has been suggested that the differences in the character of the 19S versus 7S response are due to the synthesis of the two antibodies by different cells. This is consistent with the work of Chiappino and Pernis.
showing synthesis of 19S and 7S globulins in different cells of the human spleen. However, Nossal et al. have clearly demonstrated that a single cell is capable of forming both 19S and 7S antibodies.

In addition to 19S proteins which are disulfide polymers, high molecular weight γ-globulins have been reported which dissociate into 7S units in acid. This type of macroglobulin has also been found in several patients with Sjögren’s syndrome. In these cases, in addition to the high molecular weight components (15–19S) which may resemble the peak found in classical macroglobulinemia, there are also complexes with intermediate sedimentation rates and elevation of the 7S globulins. These latter features are not found in classical Waldenström’s macroglobulinemia sera. Both the high molecular weight and the intermediate sedimenting proteins in these cases are related immunologically to 7S rather than 19S. High molecular weight isoagglutinins (approximately 19S) have also been found in colostrum and saliva which are immunologically related to γA. These antibodies have fast γ1 mobilities, are eluted in the late fractions of a DEAE column, and dissociate with the loss of biological activity on treatment with disulfide bond reducing agents. These properties resemble so closely those of the 19S γA proteins that additional (immunologic) studies are necessary to distinguish them.

Three types of γ-globulins with intermediate (9–11S) sedimentation properties have been described. One type of intermediate antibody is immunologically related to 7S γ-globulin and another to γA. Both of these have been found in sera in association with isoagglutinin activity. Intermediate γA, antinuclear factors have been described in some LE sera and saliva. A third type of intermediate antibody has been found in certain body secretions such as saliva, tears, nasal and bronchial fluids, and colostrum. This protein, although closely related to serum γA, possesses unique antigenic determinants which clearly distinguish it from the γA of serum. It is composed of multiple polypeptide chains cross-linked by disulfide bonds. However, unlike the higher polymers of γA found in normal and myeloma sera, it does not dissociate on treatment with sulfhydryl reagents in the absence of urea. Immunelectrophoresis radioautography of parotid tissue culture fluid and fluorescent antibody studies indicate its local production in salivary tissue. Recent studies suggest that γA is also selectively excreted in the saliva of cows and perhaps other species as well and that a study of saliva may be of considerable assistance in the identification of serum γA in various species.

Antigenic Heterogeneity

Important differences have been found in the γ-globulins based on a study of their antigenic properties. The following subdivisions have been identified by immunologic analysis: (1) The major immunoglobulin classes 7S, γA, and 19S which are based on antigenic determinants present on the H chains and F fragments. (2) Subgroups within a major H chain group based on H chain heterogeneity. (3) Group I and II determinants present on the L chains. (4) Subgroups within a given major L chain group due to L
chain heterogeneity.\textsuperscript{120,121} (5) Determinants which are responsible for the individual specificity of myeloma and Bence Jones proteins, macroglobulins, and of isolated antibodies.\textsuperscript{87,122-126} These are also present on the L chains and in certain instances on the H chains. (6) Genetic determinants which reside on both the H and L chains.\textsuperscript{127-130}

Within the 7S \(\gamma\) class, Grey and Kunkel\textsuperscript{114} have distinguished at least four subgroups by the use of a variety of antisera made against isolated myeloma proteins. These subgroups are based on differences in determinants present on the H chain. The H chain heterogeneity described by other workers\textsuperscript{115-117,131} may well be identical with those described by Grey and Kunkel, although additional subgroups have not been excluded in all cases.

Korngold and Lipari in 1956 divided myeloma proteins\textsuperscript{132} into three groups: one, two, and three, and Bence Jones proteins\textsuperscript{133} into two groups: A and B, on the basis of antigenic differences. Subsequently group three myeloma proteins have been identified as \(\gamma_1\). Studies by Mannik and Kunkel\textsuperscript{118} and Fahey and Solomon\textsuperscript{119} have shown that group I and II determinants of normal 7S \(\gamma\) and myeloma proteins are identical to those present on Bence Jones proteins. In a patient with both a serum myeloma protein and Bence Jones proteinuria, the two proteins are nearly always of the same group. Each of the immunoglobulin classes (7S, \(\gamma_1\), \(\gamma_1\)) as well as the Bence Jones proteins can be divided into group I and II molecules on the basis of antigenic determinants which reside on their L chains. Using \(\text{I}^{131}\)-labeled 7S \(\gamma\)-globulin and antisera made against Bence Jones proteins which were specific for either group I or II, it was demonstrated\textsuperscript{134} that approximately 60 per cent of the 7S molecules in normal serum are group I and 30 per cent are group II, and that group I and II determinants reside on separate molecules. That the group determinants are present on different molecules is further suggested by the finding that the pathologic macroglobulins and myeloma proteins are either group I or II but never both, and by fluorescent antibody studies of Bernier and Cebra\textsuperscript{135} showing that group I and II L chains are synthesized in different lymphoid cells. However, in view of the recent immunofluorescent studies of Pernis and Chiappino\textsuperscript{136} which suggest that lymph node germinal center cells can synthesize both group I and II molecules (or “mixed” molecules each carrying both group I and II) the question of the origin of the two groups in different cells remains unsettled. Mannik and Kunkel\textsuperscript{137} have shown that antibodies against restricted antigenic determinants behave like myeloma proteins in that the ratio of group I and II molecules in isolated antibody preparations may differ significantly from that present in the whole serum.

Structural studies by Putnam, of the group I and II proteins by analysis of tryptic digests of Bence Jones proteins have revealed marked differences between group I and II molecules.\textsuperscript{138} In fact, rather surprisingly, not a single peptide was identical in the two groups. However, Bence Jones proteins of the same antigenic group shared many common peptides although they also showed some differences, probably representing the individual specificity known to occur in these proteins.
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Studies of the amino acid composition, thermal, and spectrofluorometric properties, peptide maps of tryptic hydrolysates, and immunologic analysis all indicate that Bence Jones proteins are identical with the L chains of the myeloma protein from the same patient. Berggard and Edelman have shown that normal human serum and urine contain small amounts of free L chains (γ, μ) which are the normal counterparts to the Bence Jones proteins.

The Bence Jones proteins have been found to occur in several forms: monomers of molecular weight approximately 22,000, stable and dissociable dimers both with molecular weights about 45,000. The subunits in the dissociable dimers are linked by noncovalent bonds and are converted to monomers by dissociating agents such as urea, whereas the stable dimers consist of two L chains linked by a single disulfide bond.

Both γ and Bence Jones proteins according to present concepts represent unincorporated L chains of the γ-globulin molecule—the former being a by-product of normal γ-globulin metabolism and the latter resulting from the asynchronous synthesis of γ-globulin by “neoplastic” plasma cells.

Individual antigenic specificity has been demonstrated in the myeloma proteins, macroglobulins, and in isolated antibodies. Recently two different 7S antibodies isolated from a single human serum have been shown to possess individual specificity.

GENETICS OF GAMMA GLOBULINS

Intraspecies genetic differences (allotypes) in γ-globulins were reported in 1956 simultaneously by Oudin in rabbits and by Grubb in humans. The rabbit system was investigated using classical precipitin technics, whereas the human system employed Rh positive red cells coated with an incomplete human anti-Rh antibody and a serum containing rheumatoid factor. In the human system, the capacity of normal serum to inhibit the agglutination of the coated cells by rheumatoid factor was found to be genetically determined and the inhibiting factor was found to reside in the γ-globulin fraction. Sera containing inhibitor were designated Gm(+) and those lacking inhibitor Gm(-). Subsequently using different Rh coats and agglutinators of varying specificity derived from both rheumatoid and certain normal sera, eight or more inhibitory factors controlled by two or more loci have been described. A description of these factors, as well as other aspects of the genetics of γ-globulin, have been recently reviewed in detail. The genetic factors are controlled by two nonlinked loci in which the two major alleles at each locus Gm(a) and Gm(b) and Inv(a) and Inv(b) are inherited as codominant nonsex linked alleles. The Gm determinants are present only in 7S γ whereas the Inv determinants are common to all types of immunoglobulins. As a result of enzyme splitting and disulfide reduction experiments the Inv and Gm activities have been localized on different parts of the γ-globulin molecule. The Gm determinant is present on the F fragment and H chains and is unique to 7S γ-globulins, both normal and patho-
The Inv factor is localized on the S fragment and on isolated L chains and Bence Jones proteins. It appears, therefore, that different genes regulate the synthesis of the different polypeptide chains of the γ-globulin molecule. Factors controlling the H chains of γ1, and 19S γ-globulins analogous to the Gm factors for 7S γ have not as yet been reported, although they probably exist and have been tentatively designated as Gm 3 and Gm 4. However the “classical” description outlined above may require some modification in view of the recent work of Kunkel et al. suggesting the existence of at least three and possibly more loci which determine characteristics on the H chains of 7S γ-globulin. Moreover, it appears from this work that the newly described Gm (f) and not Gm (b) may be the true allele of Gm (a).

Evidence has been presented by Meltzer et al. that proteins of different Gm types differ from each other in primary structure. Fingerprint analysis of tryptic hydrolysates of F fragments of 7S γ-globulins showed specific single peptide differences between Gm (a+) and Gm (a-) fragments and also between Gm (b+) and Gm (b-) fragments. It is possible, although not proven, that these differences reside in single amino acid substitutions similar to the genetic variations in the primary structure of other proteins such as the hemoglobins.

Factors other than primary structure may, however, influence the antigenic determinants involved in genetic specificity. Recent experiments have suggested the possible importance of three dimensional structure in determining the antigenicity of a genetically controlled site and suggest that in certain instances antibodies detecting genetic variations in proteins may not react directly with the amino acids substituted in the different mutants. Rather the reaction may be with amino acid groups located at some distance from the genetically controlled area of primary structure—the latter influencing antigenicity by controlling the spatial structure of the molecule.

The γ-globulin allotypes and the formation of anti-γ-globulin antibodies against altered γ-globulins have potentially important clinical implications. Allen and Kunkel have shown that multiple transfusions may elicit the production of anti-Gm agglutinators with a specificity directed against a Gm type absent from the patient’s serum. The Snagg (serum normal agglutinator) factors found in some normal sera may also have arisen by immunization with a foreign allotype, e.g., γ-globulin administration following exposure to measles, hepatitis, etc. The Snagg factors have properties very similar to those found after multiple transfusions, one of the most striking being their specificity for a single γ-globulin allotype. The possibility exists that sensitization developing as a result of administration of a foreign allotype may lead to transfusion reactions. However, to the author’s knowledge transfusion reactions on this basis have not been reported.

Since the human fetus acquires 7S γ-globulin from its mother, the allotypes present in the newborn child are primarily those of the mother. Although it might be expected that immune tolerance would develop in the fetus to the mother’s allotypes, this is apparently not always the case. Steinberg and Wilson have found that many Snagg sera apparently result from immunization of the fetus by their mother’s γ-globulin.
Fudenberg et al. have reported the synthesis of small amounts of \( \gamma \)-globulin by the human fetus and have described a case in which anti-\( \gamma \)-globulins in the mother's serum apparently resulted from immunization by fetal \( \gamma \)-globulin. They further suggest the possibility that since Snagg factors may be of the 7S type and therefore pass the placenta that they may interact with the \( \gamma \)-globulin synthesized by the infant (if it is of the appropriate allotype) and that the transient hypogammaglobulinemia of infancy may be, at least in part, on this basis. That the transplacental passage of maternal allotypic antibodies can produce prolonged suppression of a specific allotype in the offspring has been demonstrated in the rabbit by Dray.

The Ragg (rheumatoid agglutinators) found in rheumatoid sera are generally polyvalent, and present evidence suggests that although anti-\( \gamma \)-globulin factors with genetic specificities are present in rheumatoid sera, they represent a minor component, the major specificity being directed against "nongenetic" sites. A current hypothesis consistent with these observations is that rheumatoid factors are directed against several antigenic determinants in the \( \gamma \)-globulin molecule unmasked by the spatial alterations accompanying in vivo antigen-antibody interactions.

Investigations of isolated myeloma proteins have shown that all of the genetic factors found in normal human \( \gamma \)-globulin are also present in different myeloma proteins. A single myeloma protein contains factors determined at the two genetic loci, but never the products of more than one allele (or presumed allele) even when both are present in the whole \( \gamma \)-globulin. The similarities of myeloma proteins and isolated antibodies is further extended by the work of Allen et al. showing a restricted distribution of genetic factors in certain human antibodies. It appears that in the Gm \((a+b+)\) individual, the Gm \((a)\) and Gm \((b)\) characters reside on separate molecules and that the clones of cells producing Gm \((a)\) are considerably more common than those producing Gm \((b)\) since approximately 60 per cent of the molecules carry Gm \((a)\) and only 10 per cent the Gm \((b)\) character.

By virtue of its genetic endowment a given plasma cell from a heterozygous individual should have the capability of synthesizing both allelic products, i.e., both Gm \((a+)\) and Gm \((b+)\) molecules. The apparent formation of the product of only one allele in a cell genetically capable of synthesizing two in the \( \gamma \)-globulin system (as evidenced by the myeloma data) is quite different from the abnormal hemoglobins in which the heterozygous cell synthesizes both forms of hemoglobin. For example, a single reticulocyte from a patient with sickle cell trait produces both Hb A and Hb S. Moreover, the recent immunofluorescent studies of Colberg and Dray suggest that the normal plasma cell of a heterozygous rabbit is able to produce both allelic products although in variable ratios. However, in view of the questions recently raised concerning the true alleles in the Gm system, these results are not necessarily contrary to the myeloma data. More work is necessary to clarify this important point.

A question of paramount importance is the mechanism by which large numbers of individual antibodies can be synthesized. There is considerable evidence that antibodies of different specificity differ from each other in amino
Table 2.—Diseases of \( \gamma \)-Globulin Metabolism and Their Protein Abnormalities

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Protein Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple myeloma</td>
<td>Excess 7S or ( \gamma_1 )</td>
</tr>
<tr>
<td>Macroglobulinemia</td>
<td>Excess ( \gamma_1 )M</td>
</tr>
<tr>
<td>Bence Jones proteinuria</td>
<td>Excess L chains</td>
</tr>
<tr>
<td>*H chain disease</td>
<td>Excess H chains of 7S</td>
</tr>
<tr>
<td>Hypergammaglobulinemias</td>
<td>Excess 7S, ( \gamma_1 ), ( \gamma_1 )M in varying proportions</td>
</tr>
<tr>
<td>Agammaglobulinemia</td>
<td>Deficiency of 7S, ( \gamma_1 ) and ( \gamma_1 )M</td>
</tr>
<tr>
<td>Dysgammaglobulinemia</td>
<td>Deficiency of 7S and ( \gamma_1 ) with ( \gamma_1 )M or deficiency of ( \gamma_1 ) and ( \gamma_1 )M</td>
</tr>
<tr>
<td>Beta 2A aglobulinemia in normals</td>
<td>Deficiency of ( \gamma_1 )</td>
</tr>
<tr>
<td>Hereditary telangectasia</td>
<td>Deficiency of ( \gamma_1 )</td>
</tr>
</tbody>
</table>

*H chain diseases involving \( H\gamma_1 \) and \( H\gamma_1 \)M probably exist but have not yet been reported.

acid sequence. This is suggested by the chemical and immunologic studies discussed above and by the significant differences in the amino acid composition of two different highly purified antibodies from the same animal recently reported by Koshland and Englberger.\(^{165}\) Since there are a relatively small number of loci involved, it would appear unlikely that point mutations resulting in \( \gamma \)-globulins differing in single amino acids would give rise to the necessary numbers of antibodies. The number of polypeptides varying in primary structure would be reduced if both chains were able to vary and both contributed to the combining site.\(^{59,166}\) Even if this thesis proves to be correct, there still remains the problem of the mechanism by which large numbers of L and H chains differing in amino acid sequence can be synthesized. Smithies\(^{166}\) has suggested a mechanism in which crossing over occurs, either within a single chromatid or between sister chromatids during the somatic mitotic divisions which occur in the course of the development of the immune system. This type of crossing over would presumably result in chromosomal rearrangement which would lead to the production of different polypeptide products in different antibody-producing cells; however, at present there is little definitive evidence available to support this attractive hypothesis.

CLINICAL DISORDERS

The clinical disorders associated with \( \gamma \)-globulin metabolism and the observed abnormalities in the serum proteins have been the subject of several excellent recent reviews.\(^{2,92,167,169}\) Some of these disorders and their protein abnormalities are outlined in table 2. Two general types of proliferative abnormalities are found: those in which a homogeneous protein product is synthesized presumably by a single clone of cells, and those in which a variety of clones of cells proliferate each producing an electrophoretically different \( \gamma \)-globulin.

The factor(s) leading to the marked proliferation of lymphocytes and
plasma cells in the monoclonal type of abnormalities is unknown. It is generally considered that these conditions represent neoplastic diseases. A viral etiology has been suggested by recent studies showing viral particles within some myeloma cells. The proteins produced in the monoclonal disorders may be complete as in multiple myeloma and macroglobulinemia or may represent one of the polypeptide chains of the γ-globulin molecule. The Bence Jones proteins are free L chains usually found in association with multiple myeloma or macroglobulinemias. Recently, a proliferative disorder, in which proteins related to H chains of 7S γ-globulin, has been described. Both the L and H chain diseases presumably represent the products of the proliferation of a clone of cells which for unknown reasons becomes committed to the production of one of the two types of chains it is normally capable of synthesizing. The postulated diseases characterized by the excessive production of free H chains of γ\(_1\) have not yet been described.

The diffuse hypergammaglobulinemias may be idiopathic or associated with one of many well-recognized diseases. Generally, all of the immunoglobulins are elevated although the relative concentrations may vary in different diseases. Table 3 shows in summary form the mean values for the concentrations of the immunoglobulins in a variety of clinical syndromes.

The factors which lead to the striking differences in the proportion of γ-globulins in certain abnormal sera have not been elucidated. Marked increases in γ\(_1\) are found in sera from patients with advanced cirrhosis and also in rheumatoids with high titers of rheumatoid factor, particularly those sera with large amounts of intermediate complexes.

Fudenberg, Heremans, and Franklin have postulated a genetic system analogous to the hemoglobins to explain the various forms of hypogammaglobulinemias which have been observed. They cite as evidence family studies on patients with hypogammaglobulinemia which suggest that mutations of the genes controlling the subunits of γ-globulin results in the various types of agammaglobulinemia. Defects produced by mutations at the Gm locus, which controls the synthesis of H chains of 7S γ-globulin, would result in inhibition of synthesis of 7S alone. Similarly, defects in the postulated Gm 3 and Gm 4 loci would specifically suppress the synthesis of γ\(_1\) or γ\(_1\). Decreases in two
of the three immunoglobulins would result from double heterozygosity involving two of the three gene loci and in this case a more serious clinical disorder than that produced by a single defect would presumably result. A mutation at the Inv locus leads to impaired synthesis of L chains and since these chains are common to each of the immunoglobulins there would result deficiencies in all three types of γ-globulins. This is the type of defect seen in classical congenital agammaglobulinemia. Since congenital agammaglobulinemia is sex linked, whereas the Gm and Inv loci are not, the defect in agammaglobulinemia would presumably involve a regulator gene for the Inv factor located on the X chromosome, the structural gene being located on an autosome. Somewhat analogous separations of regulator and structural genes have been described in bacteria. As pointed out by the authors, however, the hypothesis, although attractive, remains highly speculative for several reasons—a major one being that a regulator gene has not been demonstrated in a human system.

Evidence that the plasma cell deficiency which accompanies agammaglobulinemia is a result of the genetic defect in γ-globulin synthesis has recently been presented by Fudenberg and Hirschhorn. These workers showed that in vitro cultures of lymphocytes derived from agammaglobulinemic patients were able to differentiate normally into plasma cells but failed to produce γ-globulin under appropriate antigenic stimulus. Although further confirmation that the transformed cells are truly plasma cells is necessary, these findings do suggest that the defect in antibody production may result from a failure of protein synthesis rather than from a failure of plasma cell maturation.

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