atoms are produced in large numbers during the implantation and are known to be highly mobile at room temperature. Fairly convincing evidence for such a replacement mechanism has been obtained from studies by electron spin resonance techniques of silicon doped with aluminum (*15*) and also from some proton channeling studies of silicon doped with boron (*8*). Conversely, no evidence of such a replacement mechanism has been observed for any of the dopants from group V in silicon. Hence, this process might explain why only elements from group III exhibit the interstitial component.

Summary

We have illustrated how the channeling technique can be used to locate foreign atoms and also to detect lattice disorder. This technique has the advantage of looking specifically and directly at the implanted atoms. Its main disadvantage is the rather limited sensitivity. In the case of silicon, with a 1-Mev helium beam as probe, one may study a dose range of between 10^{13} and 10^{15} ion cm^{-2}. This is also the dose range of greatest present interest in ion implantation; thus this technique is useful for the analysis of implanted crystals.

We are still quite a long way from a complete understanding of the phenomenon of ion implantation. Clearly, the behavior of dopants from group III is much more complicated than that of elements from group V, and we do not yet have a detailed understanding of all the processes involved nor of their relative importance. However, the channeling technique permits one to obtain quantitative information on the location of the dopant atoms within the host lattice; this information may then be correlated with data obtained by electrical and optical studies, electron spin resonance, and other techniques.

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Immunoglobulin Structure: Variability and Homology

Amino acid sequences of immunoglobulins reflect evolutionary change and may explain antibody variability.

Frank W. Putnam

Immunoglobulins are proteins of animal origin, endowed with known antibody activity, and also include certain proteins related to antibodies in chemical structure and hence antigenic specificity (*1*). All these proteins are formed by the lymphoid cell system of vertebrates and circulate in the blood serum; they migrate electrophoretically as gamma globulins but are usually very heterogeneous, ranging into the beta globulins (*2*). Such heterogeneity precludes determination of structure by present methods of protein chemistry. In multiple myeloma, a tumor of the plasma cells which are normally a site of antibody biosynthesis, and in macroglobulinemia large amounts of homogeneous immunoglobulins are secreted into the serum, and incomplete immunoglobulins (Bence Jones proteins) may be excreted in the urine. These abnormal proteins almost invariably lack demonstrated antibody activity but are classified as immunoglobulins because of their similarity in site of synthesis, polypeptide chain structure, and antigenic specificity (*3*). The homogeneity of these abnormal proteins makes them more amenable to amino acid sequence analysis than normal γ-globulins and the natural mixture of antibodies. However, as a result of the success of sequence analysis of the abnormal immunoglobulins, considerable sequencing of normal globulins has been carried out. The abnormal proteins, though serving as a model for structural study of antibody γ-globulins, appear to differ in amino acid sequence for each patient, whereas the normal γ-globulins appear to have a mixed sequence as if they consisted of a mixture of proteins.

Because such almost infinite variability in primary structure is unique to the immunoglobulins it is thought to be related to antibody specificity. Though indirect, this is still the best evidence for the hypothesis that specific antibodies differ from each other in amino acid sequence. This is the crux of the antibody problem. How can an unforeseen agent (the antigen) impart recognition of itself into the somatic genome or express itself through the protein biosynthetic mechanism? This question leads to the paradox: Either protein structure is not invariant and is not uniquely determined by preexisting genes, or there must be already present in the genetic makeup of vertebrate animals enough immunoglobulin genes to provide for all the natural contingencies of life and the imagination of the organic chemist. To solve this problem knowledge is needed of the exact structure of immunoglobulins, and at present this is best obtainable by study of the abnormal globulins (*3*).

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Classification of Immunoglobulins

The structural relationships of abnormal globulins, normal γ-globulin, and antibodies are manifested as common antigenic determinants. Hence, by use of antiserums specific for abnormal globulins, normal human γ-globulins were classified into six major subgroups (*4*). These later were related to the polypeptide chain structures (Fig. 1) and were shown to occur generally in vertebrate species. All six subgroups and many subtypes thereof are produced by healthy individuals, but patients with plasma cell disorders usually make only a single kind, often in large amounts and invariably characteristic for the individual.

The subclassification of human γ-globulins initially depended on two facts about the immunochemistry of Bence Jones proteins: (i) that these proteins are antigenically related to normal human γ-globulin, and (ii) that there are two antigenically distinct types, kappa and lambda. By use of antiserums to Bence Jones proteins it was shown that human γ-globulins are either of κ- or λ-type and that healthy individuals produce both types in a ratio of about two to one (*4*). However, 5 to 10 percent of human light chains cannot readily be classified antigenically as either κ- or λ-type. The Bence Jones protein, which differs in structure for each patient, is either of the κ- or λ-type; it is equivalent to the light polypeptide chain of the serum myeloma globulin of the patient and is related structurally to all normal light chains of the corresponding type. Whereas the Bence Jones protein is usually homogeneous and has a unique amino acid sequence, normal light chains are heterogeneous and possibly represent as many as a thousand kinds of κ and λ light chains, each differing in amino acid sequence. Thus, amino acid sequence may be determined readily on Bence Jones proteins, but only with difficulty and much uncertainty for normal γ-globulins.

Only part of the heterogeneity of normal γ-globulins in explained by the classification into six major subgroups shown in Fig. 1. In man there are probably four additional subgroups because of the existence of the two minor classes γD and γE, and there are also four subclasses of γ heavy chains (γG1, γG2, γG3, and γG4) which differ in structure at restricted points. Allelic variants are known for both the light and heavy chains. For example, in man more than 20 known Gm genetic antigens are associated with the subclasses of the γ heavy chain (*5*), and several Inv hereditary types of κ light chains occur as well as two Oz types of λ light chains that do not segregate genetically. Analogous allotypes are found in other species. These forms of heterogeneity are independent of that associated with antigen specificity. Innumerable combinations of the many varieties of normal light and heavy chains can occur, but each abnormal immunoglobulin appears to be a unique combination of one kind of light and one kind of heavy chain. This, in addition to the fact that patients often produce large amounts of the abnormal globulins, has facilitated the burst of sequence data which has been obtained in the past 3 years and which has gone so far toward the elucidation of the general structure of antibodies.

A major breakthrough in the structural study of immunoglobulins came from recognition of their multichain structure and from dissociation of the molecules into the two kinds of polypeptide chains—light and heavy (*6–11*). The chains are so designated because of their molecular weight, about 23,000 and 50,000, respectively. In vertebrates, all three major classes of immunoglobulins (γG, γA, and γM) appear to consist of a pair of identical heavy chains and a pair of identical light chains linked together through interchain disulfide bonds (Fig. 1). The heavy chains carry the antigenic determinants characteristic of the class; they differ in structure and are designated the γ-, α-, or μ-chains. The light chains determine the antigenic type K or L and are called κ or λ, respectively. Significant homologous relationships in structure exist between κ- and λ-chains and probably also among the three kinds of heavy chains. Although the latter differ from light chains, certain general principles may govern the biosynthesis and structure of both kinds of chains, and they seem to have had a common evolutionary origin.

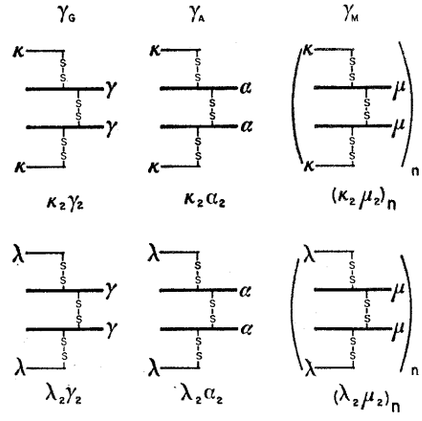

Fig. 1. Polypeptide chain structure of the three major classes of the immunoglobulins (γG, γA, and γM). Alternatively, the three immunoglobulin classes may be designated by the abbreviation Ig, that is, IgG, IgA, and IgM. The light chains are denoted κ and λ and the heavy chains, γ, α, or μ. The chain formula is given under each subgroup. Although only one disulfide bond between the heavy chains is depicted in this schematic figure, certain human γG- and γM-globulins have more than one such bond per subunit (*34*, fig. 1).

The three major classes of immunoglobulins have not yet been demonstrated in a sufficient variety of species to assure of their ubiquity. The predominant class is γG-globulin, earlier known as 7*S* γ-globulin because its sedimentation coefficient (s_{20}) equals 6.6 to 7 Svedberg units (*S*) with a corresponding molecular weight of about 160,000. The s_{20} of the γM macroglobulins is about 19*S*, for these macroglobulins are polymers of five to six disulfide-bonded 7*S* monomer units with a total molecular weight of about 10^6 (*2*). In teleosts, however, the 7*S* protein is a monomer unit of γM rather than γG. Comparable procedures are lacking for detecting γA-globulins, which may exist as 7*S* or 11*S* components. Thus, very little is known about the species distribution of γA-globulin which is present in higher abundance in secretory fluids and colostrum than in serum.

Because the molecular weight of the tetrachain immunoglobulin unit is about 160,000 in the monomeric form, it is necessary to separate the polypeptide chains or else to cleave the molecule enzymatically into reproducible fragments prior to structural study. The chain separation is accomplished by reduction with mercaptoethanol and subsequent alkylation with iodoacetamide. To minimize aggregation, the alkylated chains are dissolved in 1*N* propionic acid and are then separated by gel filtration (*6–11*). Light chain preparations from normal γ-globulin of all species are resolved electrophoretically into about ten distinct and regularly spaced components, each differing from the next by a unit electrical charge. Purified preparations of normal human κ- or λ-chains and allotypic forms of the normal chain are just as heterogeneous; thus, Cohen (*10*) has shown that there are at least 40 distinct forms of light chains in man, and probably many more. However, any of the Bence

Jones proteins from either man or the mouse usually migrate as a single band that may correspond in mobility to any of the normal bands. Preparations of light chains from specific antibodies are heterogeneous except for those from antibodies associated with chronic cold agglutinin disease or acquired hemolytic disease. This offers a possible approach for study of the structure-function relationships of naturally occurring human antibodies.

Limited Cleavage of γG-Globulin to Active Fragments

γG-Globulin can be cleaved by four methods to yield biologically active fragments; these are best defined in the case of rabbit and human γG (Fig. 2). (i) Porter (*11*) first showed that papain splits the 7*S* γG molecule into two identical 3.5*S* Fab fragments and a crystallizable 3.5*S* Fc fragment, which is a dimer of the carboxyl terminal half of the heavy chain. The Fab fragment contains the amino terminal half of the heavy chain (denoted Fd) which is still bound to the light chain through a disulfide link. If derived from an antibody, the Fab fragment retains specific combining ability for the antigen and is univalent. (ii) Pepsin cleaves γG into a 5*S* $(Fab')_2$ fragment, which contains both antibody-combining sites, and also forms oligopeptides derived from Fc. The 5*S* dimer is converted to two 3.5*S* univalent Fab′ fragments by reduction of the disulfide bond between heavy chains (*12*). (iii) If γG is mildly reduced and aminoethylated, trypsin treatment yields two 3.5*S* $(Fab)_t$ fragments that are univalent plus an $(Fc)_t$ fragment rather similar to that produced by papain (*13*). (iv) Treatment with cyanogen bromide in dilute acid produces a 5*S* $(Fab'')_2$ fragment which on reduction yields two univalent 3.5*S* Fab′′ fragments (*14*). Because the enzymatically produced Fd fragment of heavy chain is unstable, CNBr cleavage of the separated heavy chain is the preferred method for preparation of the Fd fragment.

Structure of Light Chains

Human light chains have structural features that are probably shared by the light chains of most other vertebrate species. The unique structural characteristics of light chains—also the one most difficult to explain by current genetic theory—is that both κ- and λ-chains are almost exactly divisible into an NH_2-terminal half, which is variable in amino acid sequence, and a COOH-terminal half that is almost invariant in sequence. These are designated the variable and constant regions in Fig. 3, which gives the complete amino acid sequence for one human λ light chain (Bence Jones protein Sh) and also indicates positions of identity or of variance for two other human λ light chains (Ha and Bo). In the constant region, the last 105 residues of the three proteins are identical in sequence, whereas in the variable region 53 positions are different in any two or in all three of the proteins (*15*, *16*).

Whereas proteins of the same kind within a species are believed to have a common amino acid sequence except for polymorphic variants under genetic control, the Bence Jones protein from each patient has a unique sequence. Thus far, no two Bence Jones proteins of the same antigenic type from the same species have been found to be identical. In the three human λ proteins illustrated in Fig. 3 the differences in sequence range from 40 to 53 (Table 1). In human κ-type Bence Jones proteins 73 loci of variation in the NH_2-terminal region have been discovered, and the sequence differences observed between any two proteins have ranged from 16 to 60 (Table 1). This contrasts with the single amino acid replacements found in the abnormal human hemoglobins and resembles the structural differences observed between proteins of similar kind from divergent species. Furthermore, the amino acid substitutions observed in more than 50 abnormal human hemoglobins are consistent with a one-base mutation in the corresponding codon, whereas in the above λ proteins the ratio of minimum one-base changes to two-base changes is about two to one and there are many transversions as well as transitions.

Human λ light chains (and likewise human and mouse κ-chains) may differ not only in sequence in the variable region, but also in length. Unlike the specimen illustrated, most λ-chains in man and other species begin with a blocked amino group (probably pyrrolidonecarboxylic acid, a cyclized form of glutamine, denoted PCA), whereas κ-chains generally have an unblocked

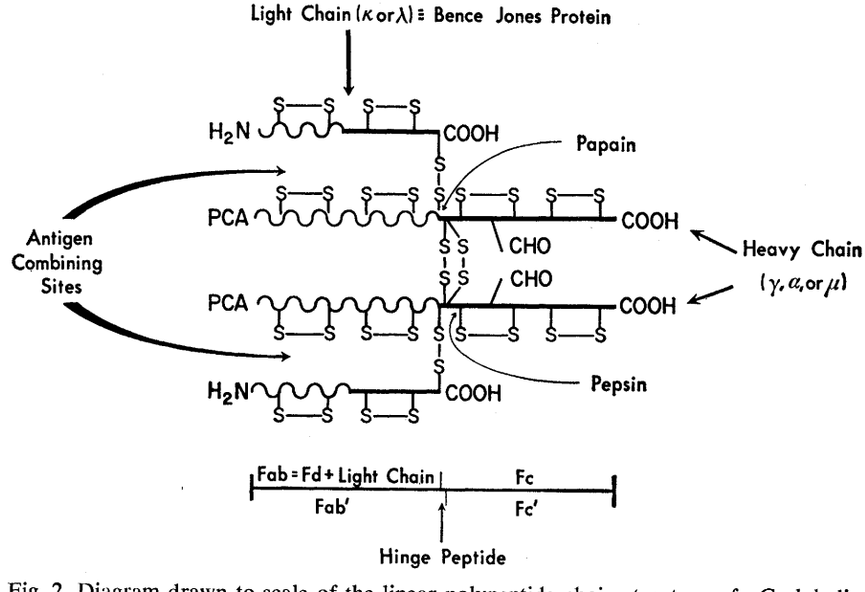

Fig. 2. Diagram drawn to scale of the linear polypeptide chain structure of γG-globulin; γA- and γM-globulins probably have an analogous structure. Two identical half-molecules are symmetrically arranged; each contains a light chain and a heavy chain. The position and number of the intrachain disulfide bonds are correctly shown in the light chains as deduced from sequence analysis of human and mouse Bence Jones proteins but are hypothetical in the heavy chains. Human γGl has two, but rabbit γG has only one heavy-heavy interchain bond at the location shown. Papain cleaves the heavy chain on the NH_2-terminal side and pepsin on the COOH-terminal side of this bond, yielding, respectively, fragments Fab, and Fc or $(Fab')_2$ and Fc′ described in the text; CHO denotes a carbohydrate prosthetic group attached at the location shown. The variable portion of the sequence is symbolized by wavy lines and the constant portion by straight lines. The location and relative length of these regions are correctly shown in light chains but are hypothetical in heavy chains. The whole Fd part of the heavy chain is certainly not variable in the fashion of the first half of the light chain, and the variability may be restricted to the first quarter of the heavy chain. For detailed sequence at the cleavage points of the heavy chain, see Fig. 5.

NH_2-terminal group, usually aspartic acid. Insertions of three amino acid residues after position 27 occur in the sequence of the human λ-type proteins Ha and Bo. At about the same place, four residues are inserted in the mouse κ-type Bence Jones protein M70 (*17, 18*) and four and six in the human κ-chains Mil (*17, 18*) and Cum (*19*), respectively. Thus, the length of light chains may vary from 213 to 221 residues, with a resulting difficulty in systematic numbering. The difficulty is resolved by using as a reference the numbering for λ-chains given for protein Sh in Fig. 3 and that for κ-chains given for protein Ag in Fig. 4, for these were first proteins of each antigenic type for which the complete sequence was reported (*15, 20, 21*).

A third feature shared by all light chains so far studied is the presence of two intrachain disulfide bonds, each forming a loop of about 60 amino acids (see Figs. 3 and 4). These are symmetrically distributed; one is within the variable half and the other in the constant half of the chain. Thus, the two halves of the molecule exhibit bilateral symmetry suggestive of two similar subunits despite the great difference in amino acid sequence of the two regions. This apparent symmetry should result in a gross similarity in conformation of the two halves of the chain and has led to the idea that light chains originated by duplication of an ancestral gene coding for about 110 amino acid residues (*22, 23*).

Another general feature of light chains, first reported by Milstein (*24*), is the presence of a cysteine residue at the COOH-terminus, which through formation of an interchain disulfide bond provides a link to the heavy chain. In most species this cysteine is the last residue in κ-chains and is the penultimate residue in λ-chains. Through disulfide bonding it may cause dimerization of free light chains excreted in the urine as Bence Jones proteins.

Although the three human λ-type Bence Jones proteins illustrated in Fig. 3 have an identical sequence for the

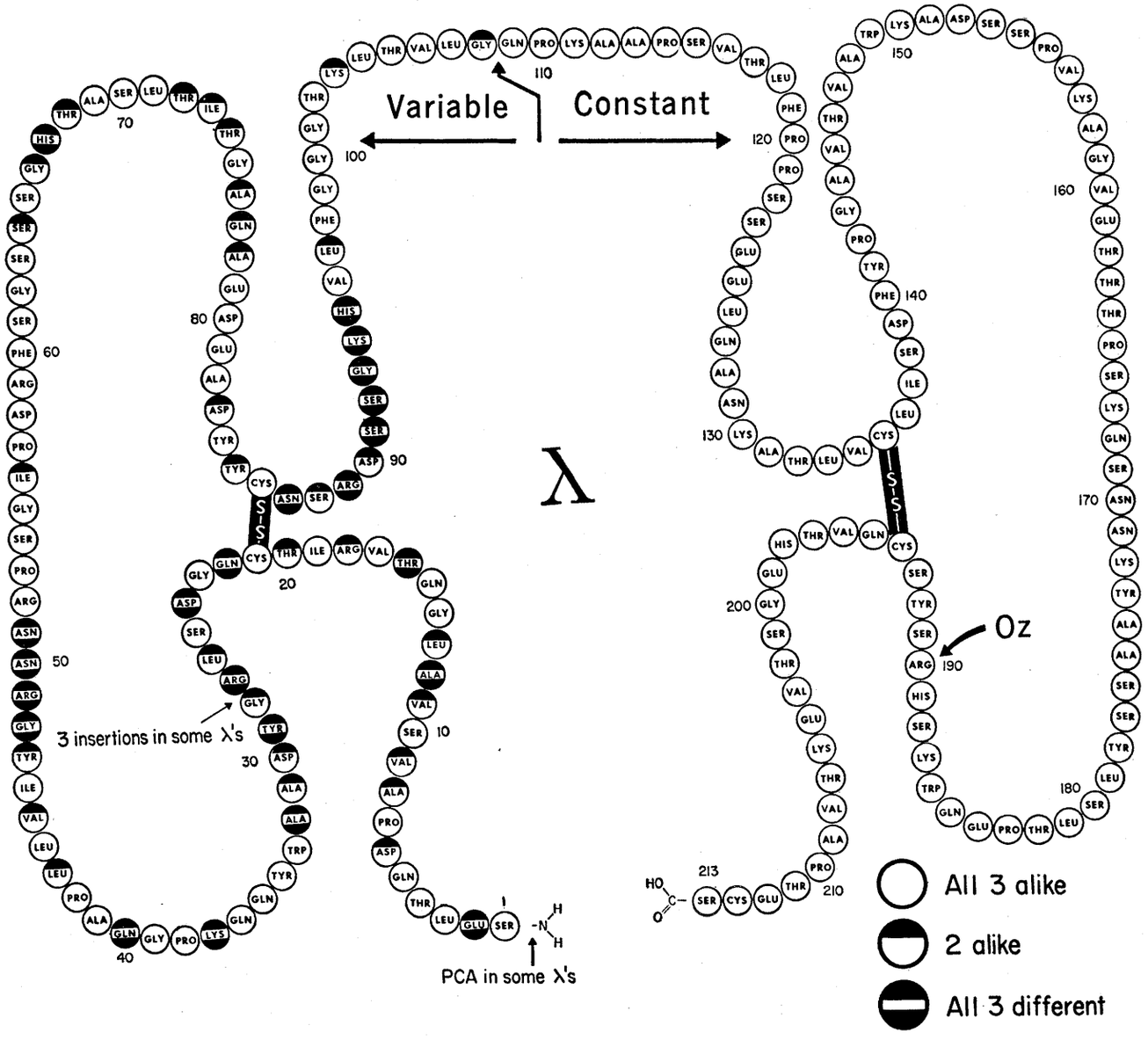

Fig. 3. Amino acid sequence of the human λ-type Bence Jones protein Sh. Positions given in white circles are identical in the human λ-type Bence Jones proteins Ha and Bo. Where the circle is black at the top, two of the three λ-chains have the same amino acid but differ from the third. All three proteins differ in positions where the circles are black at the top and bottom (*34*, fig. 4).

last 105 residues, as does a fourth (*25*), four possible loci of variation have been reported in the carboxyl half of other human λ-chains. In λ-chains of the Oz(+) serological system lysine replaces the arginine at position 190 in Oz(−) λ-chains (*26*). Ser-153 is replaced by glycine in some λ-chains (*27*), and preliminary results suggest that Ala-144 may be replaced by valine and Lys-172 by asparagine (*25*). It is unknown whether these interchanges are controlled by allelic genes or whether they represent a limited variability in sequence in the carboxyl half of λ-chains. This may provide a critical test for several hypotheses of the genetic origin of the structural variability of light chains.

Structural Variation in Kappa Chains

Like λ-chains, κ-chains differ in many positions in the NH_2-terminal portion but are essentially invariant in sequence in the COOH-terminal half. Nearly complete amino acid sequence has been reported for four human κ-type Bence Jones proteins designated Ag (*20*, *21*), Roy (*19*), Cum (*19*), and Mil (*17*). A fifth (Tew) has almost been completed (*28*), and the sequence of the variable region of one κ light chain from a human γG-globulin is known (*29*). Two mouse κ-type Bence Jones proteins have been almost completely sequenced (*17*, *18*). All these proteins exhibit the characteristics described above for human λ-chains, namely, an almost equal division into variable and constant regions, variation in chain length owing to insertions at about residue 27, the presence of two intrachain disulfide loops each of about 60 residues, and a COOH-terminal cysteine which provides a link to the heavy chain. These features are illustrated in a different alignment of the disulfide loops in Fig. 4, which depicts the amino acid sequence of the human κ-type Bence Jones protein Ag in comparison with a mouse κ-chain and a human λ-chain. In human κ-chains 73 variable loci have been identified in the NH_2-terminal portion, which ends at residue 108, but only one in the COOH-terminal half. The latter, associated with the Inv genetic factor, is located at position 191 and thus is analogous to the Oz factor located at position 190 in human λ-chains. Residue 191 is valine if the human κ-chain is Inv(b+) but is leucine if the κ protein is Inv(a+). From the number of sequence differences in the three human κ Bence Jones proteins listed in Table 1, it is evident that some κ-chains are more similar in structure than others, and thus that subtypes may exist.

Table 1. Number of amino acid differences between human kappa, mouse kappa, and human lambda Bence Jones proteins. Data are modified from Putnam *et al.* (*35*) and Dayhoff and Eck (*51*).

	λSh	λHa	λBo	κM41	κM70	κAg	κRoy	κCum
λSh	0	53	49	128	130	135	136	139
λHa	53	0	40	132	126	134	134	134
λBo	49	40	0	130	129	134	136	137
κM41	128	132	130	0	47	82	84	101
κM70	130	126	129	47	0	91	91	94
κAg	135	134	134	82	91	0	16	55
κRoy	136	134	136	84	91	16	0	60
κCum	139	134	137	101	94	55	60	0

The extent of the variation in the primary structure of κ-chains is illustrated in Table 2 by comparison of the amino acid sequence of the first 18 to 22 residues of 25 individual human Bence Jones proteins or separated light chains, all of the κ-type, for which data have been reported (*19*, *20*, *28–32*). Several of the proteins are identical in this initial sequence though differing further along the chain. Altogether for the 25 specimens, 17 different sequences have been recorded for the first 18 residues. These 17 sequences differ from each other by as many as nine residues and as few as one.

Three subtypes of NH_2-terminal sequence are evident. Type I, representing 13 specimens is like protein Ag. Type II (three examples), differs from Ag in up to 13 positions in the first 22. Type III, comprising nine specimens, differs from both of the κ subtypes I and II in almost half the positions in the sequence illustrated. There is no parallel for such remarkable differences in amino acid sequence of proteins having a similar

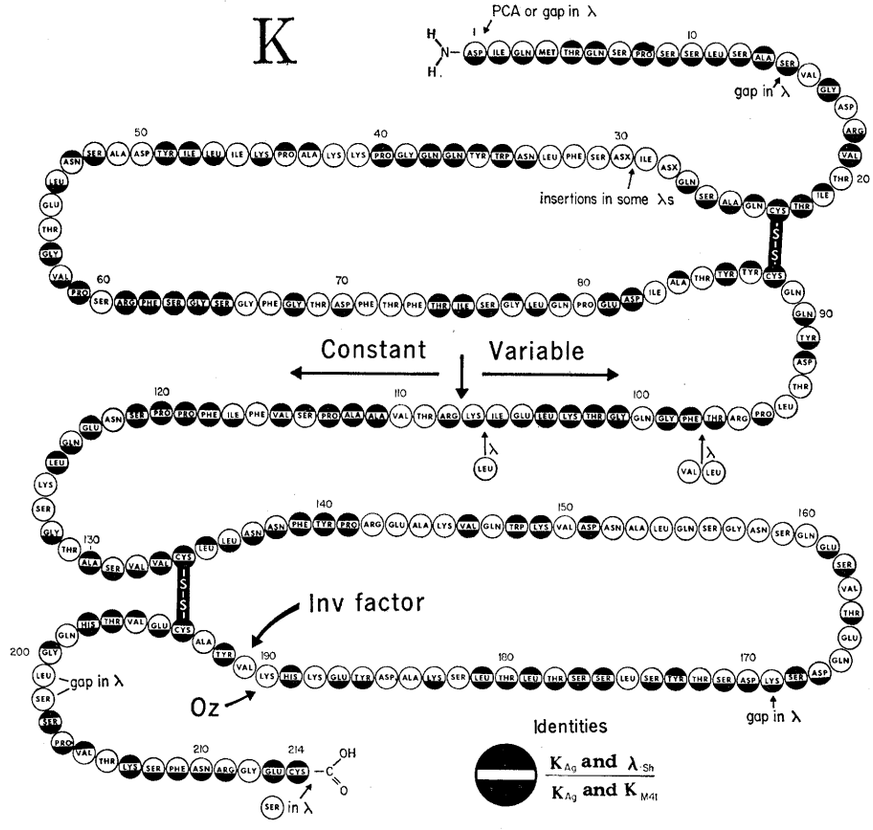

Fig. 4. Homology in amino acid sequence of human kappa and lambda light chains and mouse kappa chains. The identities in sequence of the human κ-chain Ag and the human λ-chain Sh are indicated by black in the upper half of the circle for each residue. The identities in sequence of the human κ-chain Ag and the mouse κ-chain M41 are indicated by black in the lower half of the circle. [Modified from fig. 10 (*35*)]

function and cellular site of synthesis except for polymorphic proteins controlled by separate genes.

At the bottom of Table 2 is listed the number of different residues thus far observed at each position. There are only four remaining invariant positions in the first 22 of human κ-chains, and the number of different residues at one position may be as high as four or six. This seems to rule out any simple mechanism of somatic hypermutation such as multiple recombination between just two genes. About one-quarter of the interchanges listed in the table would require a double mutation in the corresponding codons. The presence of many two-step changes indicates a high frequency of mutation but does not provide a decisive test whether the variability in sequence arose entirely by an evolutionary process resulting in many separate light chain genes carried in the germ line or whether somatic hypermutation arising from gene recombination (*33*) or some other mechanism is the origin.

Although human κ-chains may be classified into three principal subtypes, the sequence at the end of the variable region is not correlated with that at the beginning, nor is the residue at position 191 which is controlled by the Inv factor. The sequence for the last 20 residues of the variable half of six κ proteins is given in Table 3. These are of two subtypes, I and II. Proteins Ag and Roy of subtype I differ in only about nine residues up to position 96, but in five thereafter including the leucine-valine interchange at position 191. However, protein Cum of subtype II, although differing from Ag in about 56 of the first 96 positions, has only one interchange thereafter.

Table 2. The sequence is given on the top line for protein Ag (*20*, *21*). Below each position is a summary of the different residues reported in 24 other human κ-chains, from the work of Hilschmann (*19*), Whitley and Putnam (*28*), Cunningham *et al.* (*29*), Niall and Edman (*30*), Milstein (*31*), and Hood *et al.* (*32*).

Sub type		1			5				10				15				20				
I	Ag	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr
	Others		Val						Thr	Thr			Val		Leu	Arg			Ile			Ala	
			Leu							Phe													
II	Tew		Val						Leu			Pro	Val	Thr	Pro		Glu	Pro	Ala	Ser		Ser	
	Others			Leu			Thr																
III	Fr4	Glu		Val	Leu				Gly	Thr			Leu		Pro		Glu		Ala		Leu	Ser	
	Others	Lys	Met						Asx				Met										
									Ala														
Number of Different Residues		3	2	3	2	1	1	2	1	6	3	1	2	4	2	3	2	2	2	3	2	2	3

Table 3. Sequence comparison for residues 89 through 108 for five human κ Bence Jones proteins (Ag, Roy, Mil, Cum, and Tew) and for one human κ-type light chain (Eu). The sequence is given for protein Ag (*20*, *21*) but amino acid residues in other proteins that are identical to those in Ag are not shown; that is, only the nonidentical residues are listed to facilitate comparison. Data for Roy and Cum taken from Hilschmann (*19*), for Mil from Dreyer *et al.* (*17*), for Eu from Cunningham *et al.* (*29*), and for Tew from Whitley and Putnam (*28*).

Sub-type		90								100								108	191
	Ag	-Gln	-Gln	-Tyr	-Asp	-Thr	-Leu	-Pro	-Arg	-Thr	-Phe	-Gly	-Gln	-Gly	-Thr	-Lys	-Leu	-Glu	-Ile	-Lys	-Arg	---	Val
κ_I	Roy			Phe		Asn			Leu			Gly			Val	Asp	Phe						Leu
	Eu					Ser	Asx	Ser	Lys	Met					Val		Val		Gly			---	Val
	Mil	Met		Ala	Leu	Gln	Thr		Leu			Gly		Asn	Val							---	Val
κ_{II}	Cum		Met	Arg	Leu	Glu	Ile		Tyr													---	Val
	Tew	Met		Ala	Leu	Gln	Ala		Ile					Arg								---	Val
Number of Different Residues		2	2	4	2	5	5	2	5	2	1	1	2	1	1	3	2	2	3	1	2		2

Structural Relationships of Kappa and Lambda Chains

The evolutionary relationship of human κ- and λ-chains is manifested by their striking similarity in primary structure (*34*, *35*). For comparison of two proteins of different lengths, the amino acid sequences are aligned with the introduction of gaps in one or the other so as to achieve maximum identity. Each gap implies an earlier event of codon deletion in the evolutionary differentiation of the genes for the respective proteins. Maximum structural homology of the κ and λ light chains of man is obtained by the introduction of five gaps in the λ-chain and of four insertions in the κ-chain at the positions identified in Fig. 4. Some κ- and λ-chains also have insertions of three to six residues in the region of positions 27 to 32. The homology in sequence illustrated in Fig. 4 for the κ-type protein Ag and the λ-type protein Sh applies in the variable region only to the two proteins being compared, whereas in the constant region the homology in sequence holds for all human κ- and λ-chains except for substitutions due to the Inv and Oz determinants and other similar factors.

In the alignment shown, 209 positions in the two proteins are compared directly; of these 84 or almost 40 percent are identical. Thus, the degree of sequence homology of this pair of human κ- and λ-chains is similar to that exhibited by the α- and β-chains of human hemoglobin (45 percent). In addition to positions of identity there are many positions where the pairs of amino acids are chemically homologous, such as leucine and isoleucine. Conservation of the main polypeptide chain structure of human κ- and λ-chains is shown by the retention of the two intrachain disulfide bridges that determine their twofold internal symmetry. The distribution of positions of identity is seemingly random throughout the human κ- and λ-chains, and in this pairing, it is almost equally divided between the constant and variable portions. However, the number of positions of identity common to all human κ- and λ-chains in the constant region (about 40) is nearly twice the number of positions common to the variable region in the ten Bence Jones proteins (five κ and five λ) for which nearly complete sequence data are available. The number of differences in amino acid sequence between three human κ and three human λ Bence Jones proteins in pairwise combinations of κ- and λ-types is given in matrix form in Table 1. The number is remarkably constant, ranging only from 134 to 139, despite the great variation in sequence within the group of κ-chains and within the group of λ-chains. As expected if the primitive genes for κ and λ light chains diverged owing to independent mutation, there is a strong tendency for mutable (that is, variable) positions in λ-chains to coincide with those in κ-chains. This is illustrated by the proximity of the interchanges associated with the Inv and Oz factors.

Species Differences in Kappa Light Chains

Because of the infrequency of multiple myeloma in many species, extensive sequence data are available only for the κ- and λ-type Bence Jones proteins of man and the κ-type Bence Jones proteins of two tumor lines (M41 and M70) in the BALB/c strain of mouse (*36*). Mouse κ-chains resemble human κ- and λ-chains in general structural features such as a division into variable and constant regions, the presence of two large disulfide loops, the COOH-terminal cysteine for cross-linking to heavy chains, and even a difference in length because of the presence of four extra residues in M70 around position 30 compared to M41. Like the human κ protein Ag, M41 has 214 residues so these two light chains of man and the mouse can be compared directly without any insertions or deletions in the sequence. When this is done (Fig. 4), 132 positions or 62 percent of the total are identical, including superimposition of all five half-cystine residues in each chain. These two κ-type light chains of different species have an identical sequence in their first 14 residues, whereas three-fourths of the 24 other human κ-type light chains studied differ from them by one or more residues in the same length of sequence. In order to obtain the most homologous alignment with human λ-chains, deletions and insertions must be placed at the same locations in mouse κ-chains as in human κ-chains. Furthermore, the κ-chains of the two species are more closely related in amino acid sequence than are the κ- and λ-chains of man (Fig. 4 and Table 1). Thus, the interspecies homology of κ-chains is greater than the intraspecies homology of κ- and λ-chains. It is evident from Fig. 4 that all three types of light chains must be very much alike in their general conformation and three-dimensional structure. It may be concluded that these light chains, and probably all immunoglobulin light chains, originated from a common ancestral gene that duplicated and gave rise to independently mutating primordial genes of the κ- and λ-type prior to the species differentiation of higher vertebrates.

Besides the elements of size and the location of disulfide bridges, other aspects of the structure of light chains have been retained from species to species and in κ- and λ-types within man. When all the available data are compared with the alignment of Fig. 4, approximately one fourth of the residues (that is, 53) are identical in the κ-chains of man and mouse and in κ- and λ-chains of man. These residues occur twice as frequently in the COOH-terminal half of the chain as in the NH_2-terminal half. They are widely distributed throughout the chain, and except for the half-cystines involved in disulfide bonds, they appear to exhibit no particular structural function. Next to half-cystine the most frequently conserved residues are proline, tryptophan, histidine, phenylalanine, and tyrosine—all of which contain ring structures. The conservation of proline is undoubtedly related to its strong influence on the polypeptide chain conformation such as the interruption of helical segments of a chain.

Species Differences in Light Chain Classes

Proof for the existence of two classes of light chains in normal humans is based on sequence analysis of the κ- or λ-type Bence Jones proteins excreted by myeloma patients and the finding of the corresponding NH_2- and COOH-terminal sequences in pooled normal light chains. These sequences are so characteristic that they can be used to identify the classes of light chains in other species. Kappa chains generally have a free α-amino group and end with cysteine, whereas λ-chains are usually blocked at the amino end and have cysteine in the penultimate position.

Limited sequence data on the NH_2-terminal and the COOH-terminal portions of the pooled light chains of various species suggest that most higher

vertebrates and probably also sharks have two light chain classes corresponding to human κ- and λ-chains (*32*). The ratio of κ- to λ-chains varies with the species; some species, such as the horse, apparently have only a single type of light chain. The variability in sequence makes species comparison difficult at the NH_2-terminus. However, as judged from the COOH-termini, the species differences are restricted and result from the permutation of only a small number of alternative residues (Table 4). The pig has two additional residues at the COOH-terminus of a kind of light chain that Hood *et al.* (*32*) designate as κ; however, Franěk (*37*) designates this as the pi chain because its dominant amino-terminal group is alanine and thus both its NH_2- and COOH- terminal characteristics differ from human κ-type. The pig λ-chain, however, has a blocked NH_2-terminus like human λ, and there is also a high degree of similarity in sequence around the disulfide bridges of human and pig λ-chains (*37, 38*). Although 15 to 20 percent of rabbit light chains begin with pyrrolidonecarboxylic acid and are presumably of the λ-type, most begin with alanine and have three disulfide bridges, two of which seem to be in the NH_2-terminal half (*39, 40*). Like the π (or κ) light chains of the pig, this may constitute a third class of light chains. Mice make κ-chains predominantly so that a λ-type Bence Jones protein is rarely excreted. Hood *et al.* (*32*) have speculated on the selective forces involved in the production of different light chain ratios by different species.

Primary Structure of Heavy Chains

The objectives of structural study of heavy chains have been (i) to determine whether constant and variable sequences occur analogous to those in light chains; (ii) to correlate sequence differences with allotypic specificities and subclasses and with biological activity; (iii) to compare the sequences of light and heavy chains of different classes in order to discover possible evolutionary relationships and (iv) to ascertain the relation of amino acid sequence to antibody specificity.

Because complete sequences are not yet available for any heavy chain, none of these objectives has been fully attained. Detailed sequences have been given for the first 84 residues of one human myeloma γG1-chain (Daw) (*41*), and for the last 216 residues of normal pooled rabbit γ-chain (the Fc fragment) (*23*), and for a region of about 40 residues at the midpoint of both γ-chains (*7, 13, 41–46*). From these results and from partial sequence data some preliminary conclusions can be drawn. (i) Although both isotypic and allotypic differences occur in the COOH-terminal octadecapeptide of Fc in γ-chains (*47*), the constant region probably comprises the Fc portion and extends into the COOH-terminus of the Fd region (*7, 42, 45, 46*). (ii) There is a strong homology in the primary structure of human and rabbit γ heavy chains, with a somewhat weaker homology of human κ- and λ-chains with the γ-chains of either species.

Because heavy chains of the γ-type have about 440 residues, or twice as many as light chains, determination of their sequence is much more difficult. The γ-chain must first be cleaved into a small number of reproducible fragments for separate study with later reconstruction of their order in the chain.

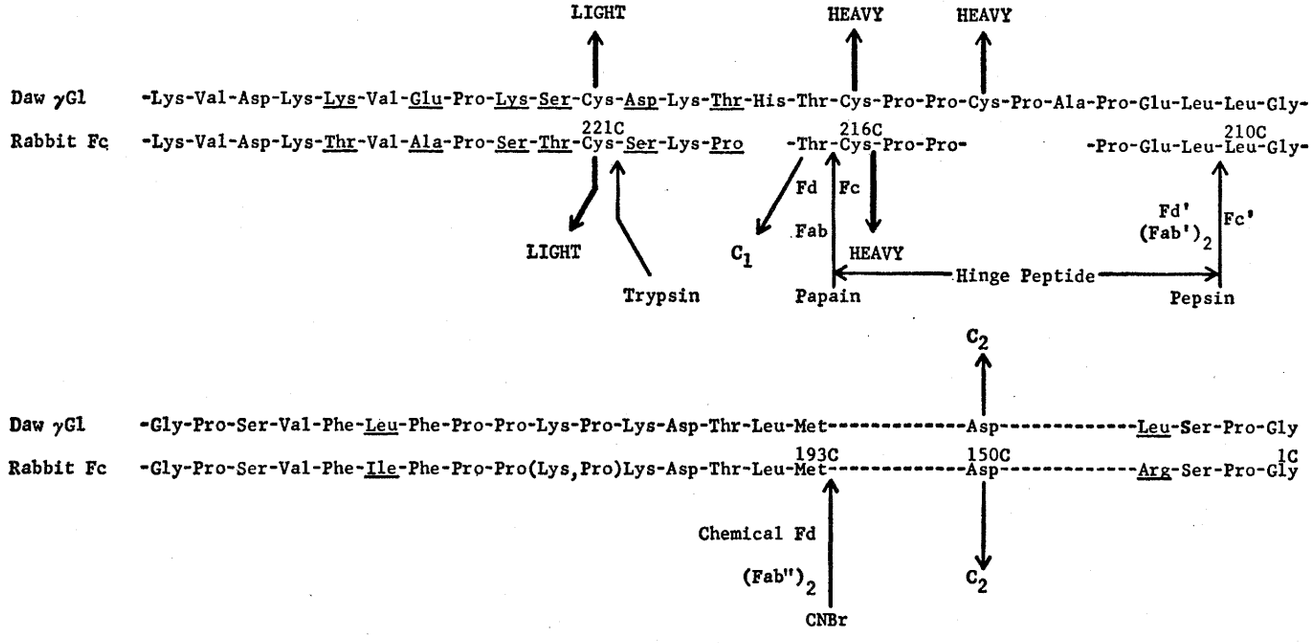

Fig. 5. Comparison of the amino acid sequence in the flexible region of human heavy chains of the γG1 subclass (Daw and Eu) and the similar portion of rabbit γ-chain. The sequence of the rabbit γ-chain is numbered beginning at the COOH-terminal residue (1C); the numbering of the human γG1-chain is uncertain. The rabbit γ-chain sequence shown is for allotypes Aa1 and Aa2. A replacement of Thr-222C by methionine has been reported for the rabbit allotype Aa3 by Prahl and Porter (*48*). Maximum homology was obtained by leaving four gaps in the rabbit chain. Nonidentical residues are underlined. The position of the light-heavy and heavy-heavy interchain disulfide bonds is shown and also the points of proteolytic cleavage by enzymes and by CNBr. The position shown for the heavy-heavy interchain bridge is definite for the human γG1-chain but tentative for the rabbit γ-chain. Based on data from various sources [Porter (*7*); Hill *et al.* (*22, 23*); Givol and DeLorenzo (*13*); Edelman *et al.* (*42*); Smyth and Utsumi (*43*); Steiner and Porter (*44*); Prahl and Porter (*48*); and Cebra *et al.* (*45*)].

Cleavage is most readily done with CNBr; the cyanogen bromide breaks a peptide link containing methionine converting the latter to homoserine which becomes the COOH-terminal residue of the fragment. Because of their different methionine distribution three human myeloma heavy chains of the γG1 class (Daw, Cor, and Eu) yielded CNBr fragments that differed in kind and number (*7, 42*). The results with rabbit γG-globulin were more complex; although all normal rabbit γ-chains seem to have three methionine residues at invariant positions in the Fc piece (*22*), the methionine at position 35 from the NH_2-terminal end (Fd piece) is missing in two-thirds of the molecules, and a methionine further on in the chain is replaced by threonine in some allotypes (*47, 48*).

The location of the intrachain disulfide bonds is not yet known exactly in any of the heavy chains. Preliminary study of rabbit γG-globulin suggests that one intrachain disulfide bond is between residues 80C and 22C in the Fc piece (where C indicates that numbering begins at the COOH-terminus). Hill *et al.* (*23*) have assumed that a second intrachain disulfide bond in the Fc piece links residues 126C and 180C. This would produce two large disulfide rings containing 55 to 60 residues analogous to the two large disulfide rings in both κ and λ light chains (Figs. 3 and 4). Two similar rings would be predicted for the NH_2-terminal half of the chain (see Fig. 2). In human γG1-chains, one such disulfide loop has been demonstrated in the first part of the Fd piece, but the remaining intrachain disulfide bonds have not yet been determined. The location and number of interchain disulfide bonds is described later.

Structural Differences in the Amino Terminus of Heavy Chains

Until recently nothing other than amino end group data had been reported for the NH_2-terminal sequences of the μ- and α-chains of the γ-globulins of any species. However, early this year Wikler *et al.* (*49*) reported the amino acid sequence of the NH_2-terminal 105 residues of a human μ-chain designated Ou. Except for one gap involving a tryptophan residue in each chain, 61 residues or 73 percent are identical in this μ-chain and the Daw γG1-chain (*41*). From these results and from other work in progress (*42*), we may anticipate that the heavy chains of each class will vary in primary structure in the NH_2-terminal region almost as much within a species as between related species, as exemplified by light chains of the κ- and λ-type. Although sequence analysis is being done on heavy chains of the γG1-type from three different human myeloma patients (Daw, Cor, and Eu) (*7, 42*), only the NH_2-terminal 84 residues of the Daw γG1-chain have been reported (*41*) at the time of this writing. However, work in progress in several laboratories indicates that there are significant differences in the size and composition of the CNBr fragments of the Fd piece of the three γG1-chains, which suggests that this area is the locus of variation in primary structure akin to that of light chains. Comparison of the sequence of the Daw heavy chain with the human λ light chain Sh showed that there are 16 identical residues in the first 50 and that similarities to human κ light chain also exist (*41*).

Still less is known about the detailed sequence of the Fd region of rabbit heavy chain. The analysis is difficult because of the variability in sequence of the normal rabbit γ-chain. This appears to be widespread beginning at the NH_2-terminus, but probably does not continue throughout the whole Fd region. In the determination of a predominant sequence in a mixture, the yield of peptides is very significant, for the methods of sequence analysis do not permit detection of variation at one position of less than 5 percent of the molecules; with yields of peptides of only 20 to 30 percent, variations could easily be missed. Five variable positions have been found in the NH_2-terminal CNBr fragment of rabbit γ-chain; yet of its 35 residues, almost two-thirds are identical with the corresponding region of the human γ-chain, including the blocked NH_2-terminal residue of pyrrolidonecarboxylic acid (*46*). Cebra *et al.* (*45*) from partial sequence data have concluded that there is a single major underlying sequence for the entire NH_2-terminal half of the normal rabbit γ-chain. They also found strong homologies with human κ- and λ-chains as well as with the rabbit Fc piece.

Table 4. Alternative residues in the COOH-terminus of the light chains of different species. Amino acid residues are listed for other species only where they differ from human light chains. From data of Hood *et al.* (*32*) and Doolittle (*40*).

Kappa							Lambda							
Human	Gly-	Glu-	Cys				Human	Val-	Ala-	Pro-	Thr-	Glu-	Cys-	Ser
Other	Asn	Asp					Other	Leu	Thr		Ser			Ala
species	Ser	Gly					species		Ser		Ala			Pro

Flexible Linkage of the Gamma Chain

The sequence at the midpoint of the heavy chain where γG-globulins of various species are cleaved by proteolytic enzymes to form the Fc and Fab fragments has been deduced for both the rabbit and human γ-chains (*7, 13, 23, 42–45*) (Fig. 5). In the rabbit γ-chain, papain mainly breaks the Thr-Cys peptide bond between positions 217C and 216C to yield the Fab and Fc fragments. This preferential cleavage is attributed to the proximity of the unusual Pro-Pro-Pro sequence which, for stereochemical reasons, must distort the molecular conformation. Because the single disulfide bridge linking the two γ-chains of rabbit γG-globulin becomes the NH_2-terminal position of the Fc fragment, the latter is a dimer whereas the two univalent Fab fragments separate as monomers. However, pepsin mainly cleaves the rabbit γ-chain at the Leu-Leu bond five residues further down the chain. This leaves the $(Fab')_2$ fragment as a dimer joined by the heavy-heavy disulfide bond. After reduction and carboxymethylation of the latter, incubation with papain releases a series of overlapping peptides containing the sequence Thr-Cys-Pro-Pro-Pro-Glu-Leu, the last five residues of which are known as the "hinge peptide." The carbohydrate prosthetic group C_1, containing galactosamine is attached to the threonine at position 217C in about 35 percent of the heavy chains of rabbit γG, whereas the carbohydrate prosthetic group C_2 containing glucosamine is attached to the aspartic acid residue at

	135							140							150
Human λ	Cys	Leu	Ile	Ser	Asp	Phe	Tyr	Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp
Human κ	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp
Mouse κ	Cys	Phe	Leu	Asn	Asn	Phe	Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp
Rabbit F_c	Cys	Met	Ile	Asp	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ser	Val	Gly	Trp	Glu	Lys	Asp

Fig. 6. Comparison of homologous segments of the amino acid sequence of human and mouse light chains and rabbit Fc heavy chain fragment. The numbering system refers to the human λ-chain. Underlined residues are identical in two or more of the chains. Data obtained from Wikler *et al.* (*15*), Titani *et al.* (*20*), Dreyer *et al.* (*17*), and Hill *et al.* (*22*).

position 150C and appears to be present in all rabbit γ-chains. It is uncertain whether the distribution of galactosamine is asymmetric or whether it is symmetric so that it is on both heavy chains in some molecules and absent from both in others. The $(Fab'')_2$ fragment ends in homoserine, the CNBr product of methionine; its two heavy chain pieces have the first 24 residues of the papain-produced Fc and are joined by the bond between heavy chains at Cys-216C. Thus, the order of size of the Fab fragments produced by the various methods is: CNBr > pepsin > trypsin. The susceptibility of the heavy chain to cleavage by various agents near the midpoint suggests that this segment is exposed and is perhaps flexible; it could be the swivel point in the Y-shaped model of antibody γ-globulins deduced from electron micrographs (*50*).

Although the points of cleavage of the human γG1-chain by proteolytic enzymes are less well defined, it is probable that they are identical with those of the rabbit γ-chain since similar fragments are obtained, and the sequence in the flexible region is closely homologous. Apart from the four gaps inserted in the rabbit chain to permit the most homologous alignment, the sequence is identical in about three-fourths of the residues shown, and is nearly identical from the beginning of the Fc region (*43*). This seems to be a region of stable sequence because three human myeloma globulins (Daw, Cor, and Eu) of the γG1 subclass have the same heptadecapeptide sequence around the hinge peptide (*7, 42*).

Amino Acid Sequence of Fc Fragment; Carboxyl Terminus of Gamma Chains

From comparison of the sequence of the 216 amino acid residues of the rabbit Fc fragment and the 214 residues of the human κ Bence Jones protein Ag (with insertion of gaps to give an alignment with the maximum number of identical and similar residues), Hill *et al.* (*22, 23*) found 42 identical residues at the same position; this is equivalent to 25 percent of the positions compared. An equal number of residues in the two apparently diverse chains were chemically homologous. This striking homology in sequence suggested an evolutionary relationship between the light and heavy chains of immunoglobulins.

An example is given in Fig. 6, which shows the consecutive sequence of 18 residues beginning with half-cystine in each of the four chains. Seven of the columns contain identical residues; in others there are identities for two or three of the chains for a total identity of 62 percent. Although the similarity in sequence is not as strong throughout the four chains, other segments do have a high degree of homology (*22, 23, 34, 35*). There is also a weak homology within the rabbit Fc fragment and within the mouse and human light chains, which suggests that all of these chains arose by duplication of a primitive gene coding for about 110 amino acids. Tentative phylogenetic trees for the immunoglobulins have been drawn on the basis of this structural homology (*22, 23 51*).

Sequence data have been reported only for fragments of the COOH-terminal portion of the heavy chain of other species. Several peptides derived from the Fc region of the human myeloma globulin Eu show strong homology with

Table 5. Differences in the COOH-terminal sequences of human γ-chains of differing isotypes and allotypes and the γ-chains of other species. The sequence given on the top line is for two human myeloma globulins of subtypes γG1 and γG2 and is also the main sequence of normal γG-globulin. Positions are indicated for the other immunoglobulins, only where the sequence differs from human γG1 and γG2. The two sequences for human γG3-globulin are for myeloma proteins that differ in their Gm allotype. The amide positions in the bovine γG-globulin are uncertain.

		210	211	212	213	214	215	216	Reference
Human	γG1, γG2	Met	His	Glu	Ala	Leu	His	Asn	(7,41)
	γG3	Arg	Phe						(47)
	γG3	Arg	Tyr						(47)
	γG4	His	Tyr					Leu	(47)
Horse	γG					Val	Lys		(57)
	γG(T)	Val	Glu			Asn	Val	His	(57)
Rabbit						Ile	Arg		(22,23)
Bovine	γG1					Thr	Lys	Ala	(58)
	γG2			Met		Thr	Lys	Ala	(58)

corresponding peptides from rabbit Fc (*42*). For example, one 12-residue peptide from Eu has a sequence identical to rabbit Fc positions C109 to C120, and the glycopeptides of the two Fc pieces differ by only a single replacement. Considerable homology in the Fc regions of the γ-chains of different species can be predicted from the comparative data that are available on the COOH-terminal nonadecapeptide sequence of the four subclasses of human γ-chains, rabbit γ-chains, two varieties of horse γ-chains and two of bovine γ-chains (Table 5). The resemblance in sequence is remarkable; of the 19 positions ten are identical in all ten γ-chains. The order of difference is about the same between allotypes as between species. In fact, the most variant sequence shown is that of the horse γG(T)-chain which may represent a different class than horse γG-chain. The latter two differ more from each other than horse or rabbit γG or bovine γG1 do from human γG1 and γG2. These phylogenetic relationships are strong evidence that γ-chains diverged from a common ancestral gene.

On the other hand, human heavy chains of the μ- and α-types differ significantly in structure from γ-chains in their COOH-terminal sequence. The μ-chain has the sequence -Met-Ser-Asx-Thr-Ala-Gly-Thr-Cys-Tyr-COOH (*49, 52*) The α-chain ends in the same tripeptide *(53)*, but in the region indicated neither has a single residue in common with any of the four subclasses of human γ-chains. This suggests that differentiation of the genes for γ-, α-, and μ-chains occurred very early in evolution.

Summary

Antigenic and structural study of homogeneous immunoglobulins produced in human disease has enabled the classification of the normally heterogeneous system of γ-globulins. The division of immunoglobulins into three major classes (γG, γA, and γM) based on the kind of heavy chain and into two antigenic types (κ and λ) determined by the light chain seems to be general for all species; however, the relative distribution of each of the six groups may vary with the species and is affected by the age and immune state of the animal. Isotypic differences in structure give rise to subclasses present in all normal animals of the same species, and allotypic differences among individuals produce a genetic polymorphism within a species.

The Bence Jones proteins excreted by patients with multiple myeloma correspond to light chains but differ individually in their amino acid sequences (*16, 21, 54*). Both κ- and λ-chains in man and κ-chains in the mouse have many loci subject to variation in the NH_2-terminal half of the chain but have only one or a few inherited variations in the COOH-terminal half. A similar structural division of heavy chains is suggested by partial sequence analysis of the normal γ-chain of the rabbit and of the γG1-chain of human myeloma patients. Because this variability in structure is limited to the portion of the molecule containing the antigen-combining site, it must give rise to antibody diversity and probably is related to antibody specificity.

The nature of the mechanism which produces the variability in amino acid sequence is unknown. There are two principal, alternate theories, namely (i) there are many genes for the κ- and for the λ-types of light chains and likewise for the three main classes of heavy chains, and (ii) genes for immunoglobulin light (and heavy) chains undergo somatic hypermutation. In the first theory (the multiple germ line theory), some authors assume that many separate genes code for the variable part of the chain and only a few allelic genes code for the constant part (*17, 18*). In the alternative hypothesis, the somatic hypermutation has been attributed to an excision and faulty repair mechanism (*55*) or to several possible kinds of recombinational events between two genes [the master and the scrambler in the Smithies hypothesis (*33*)], or among a small number of genes that differ only in the variable part (*56*). Neither the germ line theory nor the somatic hypermutation theory are readily subject to experimental testing; neither successfully explains the genetic and evolutionary stability of the constant portion of the chains including the allotypic variations.

Evolutionary relationships among light and heavy chains of the same and different species are manifested by the homology in primary structure which persists, despite the variability in amino acid sequence even within the same type of chain from one species. Large disulfide loops containing about 60 amino acids are characteristic features of both the light and heavy chains; these loops dominate the conformation of the chains, impart internal symmetry and seem to have important roles in antibody function. Comparative structural study suggests that the genes for both light and heavy chains evolved from a common ancestral gene coding for about 110 amino acid residues.

Subsequent to the submission of this paper, Gottlieb *et al.* (*59*) reported the sequence of the first 96 residues of the human γG1 heavy chain Eu. The latter was identical to the γG1 heavy chain Daw in only 26 of the 82 positions compared. Of these, 24 are also identical in the Ou μ-chain. These results indicate that the amino acid sequence of the NH_2-terminus of heavy chains is highly variable, like that of light chains, but may not be specific for the class of heavy chains. The latter conclusion is also supported by the report of Bennett (*60*) who found differences in the NH_2-terminal pentapeptide sequences of four human μ-chains.

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Nutrition and Learning

Inadequate nutrition in infancy may result in permanent impairment of mental function.

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It has long been scientifically acceptable and, in some circles, even fashionable to ascribe many behavioral characteristics of the older child and adult to conditioning experiences received during infancy and perhaps prenatally. More recent data have indicated that some of these conditioning factors, rather than being psychosocial in nature, have a biochemical basis. Alterations of the biological and physical environment produce profound and lasting disturbances of the anatomical, chemical, and thus developmental and behavioral pattern of the organism. This course of events has been aptly termed "biological freudianism" by Dubos *et al.* (*1*).

The best studied aspect of biochemical conditioning is nutrition. In most areas of the world malnutrition in early life is directly or indirectly responsible for more deaths among children than all other causes combined; recent evidence has indicated that deficiencies in nutrition not only affect physical growth but may produce irreversible mental and emotional changes. Many aspects of these long-term effects of malnutrition have been studied in animals (*2*); from these experimental data a series of hypotheses relating to human development has been proposed.

Nutrition and Physical Growth

Numerous observations in animals dating back many years indicate that malnutrition retards physical growth (*3*); if growth is suppressed for a sufficiently long period during a critical phase in the early weeks or days of life, subsequent supplementation of the diet, while initially enhancing the rate of growth, usually does not permit the animal to attain its normal mature size. The same sequence of events has been demonstrated to occur in children who had suffered from severe malnutrition during early life.

To a considerable extent, biochemical development parallels this suppressed physical development in both children and experimental animals. Maturation of a variety of biochemical processes is delayed; thus, malnutrition results in inhibition of the biochemical maturation of the organism, and may, under certain conditions, produce retrogressions to earlier functional patterns. In malnourished children, such widely different measurements as water distribution, fat absorption, concentrations of plasma lipids and cholesterol, and excretion of creatinine approximate corresponding values observed in well-nourished younger children of the same height and weight (*4*). Furthermore, the metabolism of phenylalanine to tyrosine is depressed in malnourished older infants. Such patients show an abnormally high ratio of phenylalanine to tyrosine in the blood plasma and excrete excessive quantities of phenylalanine in the urine;

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