shows this not to be the case; species found in shallow-water deposits in the Miocene and Pliocene are not presently found in abyssal depths although they still live in shallow waters. Likewise, one would expect deep-water assemblages in the Miocene and younger Tertiary to be largely or completely different from the post-Pleistocene. A sample of *Globigerina* ooze of early Miocene age was dredged at a depth of 847 m, and many of the species presently restricted to the abyssal fauna also occur only in the early Miocene deep-water assemblage. Thus, our evidence indicates that the abyssal fauna is not Recent in origin, and that time to develop the diverse faunas was available.

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Macroglobulin Structure: Homology of Mu and Gamma Heavy Chains of Human Immunoglobulins

Abstract. *The amino acid sequence of fragments obtained by cyanogen bromide cleavage of the mu-chain of a human γM-globulin is homologous to the NH_2-terminal sequences of the gamma-chain of human and rabbit γG-globulins and is related to that of human light chains. This supports the hypothesis that light and heavy chains evolved from a common ancestral gene.*

Immunoglobulins embrace antibodies and proteins related to antibodies in having a tetrachain structure composed of a pair of heavy and a pair of light polypeptide chains, and also include Bence Jones proteins (free light chains). The three major classes of immunoglobulins, γG, γA, and γM (or alternatively, IgG, IgA, and IgM), are demarcated by their heavy chains (γ, α, and μ, respectively); each class is divided into two antigenic types (K or L) based on the presence of a pair of kappa or of lambda light chains. Normal κ and λ light chains of all species are heterogeneous owing to variability in amino acid sequence of the NH_2-terminal half (about 110 residues); in contrast, the COOH-terminal half (105 to 107 residues) is invariant in sequence except for single amino acid substitutions, some of which are apparently allelic (*1*). This conclusion is based on complete amino acid sequence analysis of some ten individual human κ- and λ-type Bence Jones proteins excreted by patients with multiple myeloma or macroglobulinemia (*2–6*) and of the κ-type Bence Jones proteins from two mouse plasmacytoma strains (*3*). It is supported by partial sequence analysis of the NH_2-terminal and COOH-terminal peptides of the κ- and λ-type light chains of many species (*7*). A similar bipartite structure has been proposed for heavy chains (*1*); the latter are composed of somewhat more than 400 amino acid residues, and can be cleaved enzymatically (*8*) into an NH_2-terminal (Fd) and a COOH-terminal (Fc) fragment of approximately equal size. Although the Fd-fragment is believed to have the variable, and the Fc-fragment the constant sequence, this has not yet been established because the maximum length of sequence published comprises only the NH_2-terminal 84 residues of one human γ-chain from a myeloma patient designated Daw (*9*) and the COOH-terminal 216 residues of the γ-chain from pooled, normal γG-globulin of the rabbit (*10*).

We report here the complete sequence of three fragments (F1, F2, and F3) apparently comprising the first 105 residues of the NH_2-terminal portion of a μ-chain from a pathological human γM-macroglobulin designated Ou (Fig. 1), and also the sequence of two smaller fragments from the COOH-terminal (Fc) portion of the μ-chain (Fig. 2). When only one gap is placed in each chain, 61 residues in the first 84 (or 73 percent) are in identical positions in the human γ-chain Daw and the human μ-chain Ou, and the two chains are equally homologous to the NH_2-terminal F1 fragment comprising the first 35 residues of the normal rabbit γ-chain (*11*). Evolutionary relationships of heavy and light chains are suggested by these similarities in sequence of the three heavy chains and also by similarities to κ- and λ-chains.

The μ heavy chain of the κ antigenic type γM-globulin from patient Ou was prepared by mild reduction with dithiothreitol or β-mercaptoethanol to break the interchain disulfide bonds and subsequent alkylation with iodoacetamide. The κ light chains and the μ heavy chains were separated by gel filtration on Sephadex G-100 and three major peaks resulted. The heavy chain was cleaved by reaction with CNBr for 24 hours in 70 percent formic acid; this breaks the peptide bond on the carboxyl side of methionine and converts methionine to homoserine. The fragments formed by CNBr were fractionated with Sephadex G-100 and purified by repeated gel filtration or ion-exchange chromatography. The purified fragments were completely reduced and were aminoethylated with ethylenimine or were carboxymethylated with iodoacetamide to break intrachain disulfide bonds.

In two cases, reduction and aminoethylation yielded additional fragments. Nine fragments formed by CNBr were defined for the Ou heavy chain; altogether these accounted for the total amino acid content of the untreated μ-chain. The largest fragment was composed of about 130 amino acids (Sephadex peak 2); it accounted for the bulk of the carbohydrate in the original γM-globulin and had a COOH-terminal homoserine residue. Two homoserine-containing units in peak 1, each composed of about 100 residues, appeared to be linked by an intrachain disulfide bond before reduction. The remaining fragments were smaller and varied in size from 4 to 50 amino acid residues. Their amino acid sequence, as determined by use of the Edman degradation method on the tryptic and chymotryptic peptides, is presented in this report. Identical fragments were also obtained by cleavage of the whole γM-globulin with CNBr followed by

reduction, alkylation, and gel filtration.

Three cyanogen bromide fragments of the μ-chain, F1, F2, and F3, were present in a single fraction (peak 3, Sephadex G-100). Fractions eluted later contained FX, FY (17 residues), and FZ (eight residues). Fragment FX is the smallest containing homoserine, and it has a high content of a tyrosine-like component that has not yet been fully characterized.

After reduction and aminoethylation of peak 3 fragments F1 and F3, each containing one residue of aminoethylated cysteine, were resolved by gel filtration. This indicated the presence of a disulfide bridge between F1 and F3 which was demonstrated directly by isolation with the diagonal technique (*12*) of disulfide-bridged peptides from a peptic digest of the whole Ou macroglobulin. One peptide involved in the bridge had the sequence Thr-Cys-Thr-Phe-Ser-Gly (*13*) later identified in F1 (Fig. 1) and the other had the sequence Tyr-Cys-Ala-Arg-Val-Val-Asx, which is present in F3 (Fig. 1). Disulfide-bridged peptides having the same sequences were also isolated from a tryptic digest of F1 and F3 made before reduction and alkylation. Thus, Cys-22 and Cys-97 in Fig. 1 are linked by a disulfide bond.

By sequence analysis of the products of tryptic digestion of the *S*-aminoethylated fragments F1, F2, and F3 and of the chymotryptic peptides obtained from the *S*-carboxymethylated fragments, we determined the complete amino acid sequence of these three portions of the μ heavy chain. The overlapping chymotryptic peptides defining all positions within each fragment except 39 through 49 and 66 through 69 have been characterized. The order of a few tryptic peptides containing these positions has been arranged in Fig. 1 by analogy to the NH_2-terminal portion of the human γ-heavy chain Daw (*9*).

As yet, we have no direct proof for the order of the CNBr fragments of the μ-chain, but we have made a tentative assignment for five of the nine fragments including the placing of F1, F2, and F3 as the NH_2-terminal segment. Since κ- and λ-light chains are homologous in amino acid sequence (*1*), we have assumed that γ- and μ-heavy chains are also homologous. Fragments F1 and F2 are remarkably similar in length and in sequence to CNBr fragments 2b and 4, respectively, of the Daw γ-chain (Fig. 1). Fragments F1 and 2b each contain 34 residues, of which 27 are identical at the same position including the COOH-terminal methionine. Fragments F2 and 4 each contain 50 residues, of which 34 are identical at the same position provided a gap is inserted at one tryptophan residue in each fragment. Thus, for the sum of 84 residues in F1 + F2 and in 2b + 4, 61 residues or 73 percent are identical in this segment of these two human μ- and γ-chains. Many other positions are chemically homologous, such as the replacement of Lys-11 in Ou by Arg-11 in Daw.

Like most human γ-Chains (*8, 9*) and μ-chains (*14*) the Ou μ-chain is blocked at the NH_2-terminal position, that is, the α-amino group does not react with fluorodinitrobenzene or in the Edman reaction, probably owing to the presence of pyrrolidone-carboxylic acid, a cyclic form of glutamine denoted PCA. By ion-exchange chromatography of a subtilisin digest of the whole γM-globulin, we isolated the NH_2-terminal peptide as a ninhydrin-negative tetrapeptide, the sequence of which was found to be PCA-Val-Thr-Leu. This is identical to the NH_2-terminal sequence of the Daw γ-chain. Since we found the

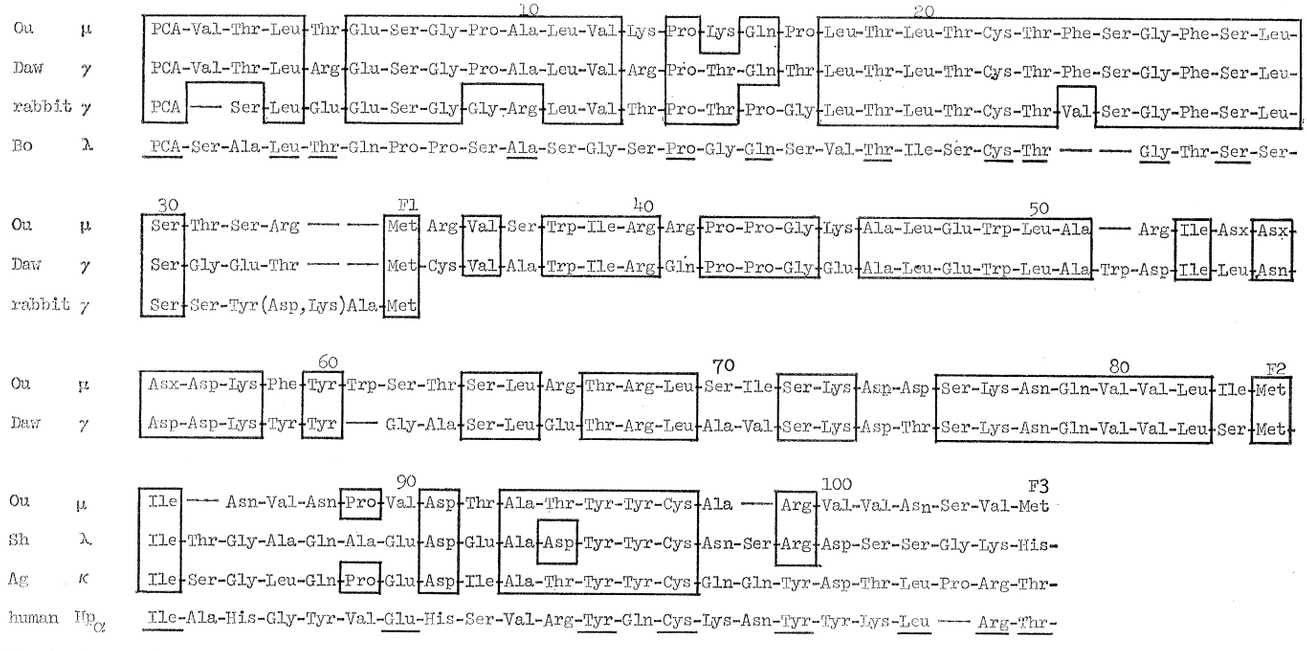

Fig. 1. Comparison of portions of the NH_2-terminal sequence of the human μ-chain Ou (105 residues), the human γ-chain Daw (84 residues) (*9*), and normal rabbit γ-chain (35 residues) (*11*). Identical residues in all three or any two proteins are included in boxes. The three heavy chains are compared for the length of the fragment removed from the NH_2-terminus by CNBr cleavage. The first line also gives the NH_2-terminal sequence of the human λ-chain Bo [Putnam *et al.* (*5*)]; the last lines give the sequence of residues 73 through 95 of the human λ-chain Sh [Putnam *et al.* (*5*)], residues 75 through 97 of the human κ-chain Ag [Putnam *et al.* (*2*)], and 22 through 43 of the α^1-chain of human haptoglobin (*18*). Residues that are underscored in the λ- or α^1-chains are identical to those at corresponding positions in one or more of the μ- or γ-chains. Gaps have been introduced in the sequences to secure the maximum number of identities. Fragments F_1, F_2, and F_3 of the μ-chain have been ordered by analogy to the human γ-chain (see the text). Cys-22 in F1 and Cys-97 in F3 are linked by a disulfide bond.

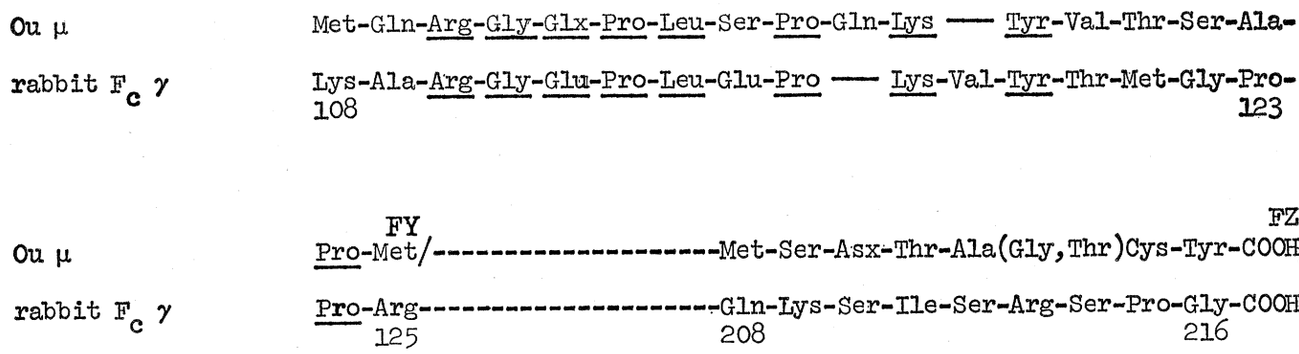

Fig. 2. Comparison of fragments FY and FZ obtained by CNBr cleavage of the μ-chain of the human γM-globulin Ou to portions of the Fc fragment of normal rabbit γ-chain (*10*). Identical residues in the two heavy chains are underlined. Dotted lines represent schematically the portion of the amino acid sequence not shown. The rabbit γ-chain is numbered from the NH_2-terminus of the Fc fragment.

same sequence in peptides from both the chymotryptic and tryptic digests of F1, we concluded that F1 was the NH_2-terminal fragment. Fragment F2 was assigned to the COOH-terminal side of F1 because of the strong homology to the Daw γ-chain (Fig. 1). In the absence of sequence data on γ-chains beyond residue 84, F3 was tentatively assigned to the carboxyl side of F2 on the basis of homology to the disulfide bridge structure of light and heavy chains. The variable and constant portions of the κ- and λ-chains of man and the κ-chains of the mouse have an intrachain disulfide loop containing 60 to 70 residues (*1–6*), and two such loops are present in the Fc fragment of the rabbit γ-chain (*10*). This would correspond approximately to the disulfide bridge we found in the μ-chain between Cys-22 in F1 and Cys-97 in F3.

Fragment FZ from peak 5 (Fig. 2) was identified as the COOH-terminus of the μ-chain because of the absence of a homoserine residue and because carboxypeptidase A released tyrosine from the whole μ-chain. This agrees with the report that Ala-Gly-Thr-Cys-Tyr is the COOH-terminus in human μ-chains (*15*). The cysteine residue in this sequence was recovered in the *S*-carboxymethylated form, indicating that it was involved in an interchain disulfide bond that was selectively cleaved and alkylated during chain separation. Fragment FZ does not have the reported sequence adjacent to the disulfide bonding of the heavy and light chains of a γM-globulin; and it is not analogous to the proline-rich sequences adjacent to the heavy-heavy interchain bonds of human and rabbit γ-chains or to the sequences adjacent to the intrachain disulfide loops in the Fc portion of human γ-chain (*16*). Hence, the unique cysteine residue of μ-chains is probably involved in the intermolecular disulfide bridge linking five 7*S* γM monomer units to form polymeric 19*S* macroglobulins. Indeed, when CNBr fragments were prepared from the whole γM-globulin, the COOH-terminal fragment was not eluted at the position of FZ, presumably because it was in the dimeric form. Although human μ-chains appear to have an identical sequence for the COOH-terminal pentapeptide, there is no similarity to the corresponding portion of either human or rabbit γ-chains. This may reflect a different length for the μ-chains.

Fragment FY isolated from peak 4 has strong homology in sequence to residues 108 to 125 of the Fc region of rabbit γ-chain (Fig. 2). By introduction of one gap in both proteins, nine residues can be aligned as identical. As suggested in Fig. 2 by the analogy to rabbit γ-chain, FY and FZ may be separated by 80 to 100 residues in the Fc region of the human μ-chain, and the intervening region may represent an area of nearly constant sequence in the Fc portion of human μ-chains.

By analogy to human light chains, the NH_2-terminal sequence of heavy chains of identical class should be highly variable; yet, counting gaps, two human heavy chains of different class, μ and γ (Ou and Daw) have only 23 differences in sequence in the first 84 residues whereas the three human λ-chains for which we have reported the complete sequence differ in from 27 to 40 residues in the same length of NH_2-terminal sequence (*5*). Likewise, human κ-chains of different subtype such as Ag and Cum differ in about half the residues of the first 84 in their NH_2-terminal sequence (*2, 4*). If light chains of identical type differ almost twice as much from each other in a given length of sequence as heavy chains of the γ- and μ-classes differ from each other, it would be surprising if heavy chains of identical class, either μ or γ, exhibit variability in sequence in their first 84 residues, comparable to that found for light chains. However, subsequent to the submission of this paper Gottlieb *et al.* (*17*) 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 great similarity of the NH_2-termini of the human μ-chain and the rabbit γ-chain is shown in Fig. 1. To achieve maximum homology to rabbit γ-chain the same gaps have to be made in the human μ- and γ-chains; then, 60 percent of the sequence of the three F1 fragments is identical. In this region, the two classes of human heavy chains are somewhat more closely related than are the γ-chains of the two species. This is in contrast to the case for light chains where the κ-chains of man and mouse are more alike (60 percent identity) than the κ- and λ-chains of man (40 percent identity) (*1*). Functional and conformational limitations on acceptable mutations may have imposed restrictions on the diversity in sequence of the heavy chains. Alternatively, the differentiation of heavy chains, unlike that of light chains, may have followed interspecies differentiation. For this reason, less variability in sequence may exist in the

heavy chain classes than in the light chain types.

The hypothesis of a common evolutionary origin of light and heavy chains is supported by the sequence data for human μ-chains. Eight of the first 27 residues of a typical human λ-chain are identical with corresponding positions in the three heavy chains in Fig. 1; other positions are identical with those in one or more of the heavy chains. A similar homology is shown when corresponding portions of human λ- and κ-chains are compared to the sequence of the F3 fragment of the human μ-chain (Fig. 1). In support of the suggestion (*18*) that haptoglobins also evolved from a primitive light chain precursor, a weak homology exists when the sequence of the human haptoglobin α^1-chain is compared to that of the κ-, λ-, and μ-chains.

More structural data on γ-, α-, and μ-chains of several species will be required before the phylogenetic and evolutionary considerations proposed above can be verified. Complete sequence data on individual γ- and μ-chains of the same species are needed to fix the location of the variable and constant portions of heavy chains and to ascertain the extent of the variability in sequence.

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7 October 1968

Photosynthesis: Temperate and Tropical Characteristics within a Single Grass Genus

Abstract. *Leaves of two subgenera of* Panicum *differ in photosynthetic physiology and bundle sheath characteristics. Species of the subgenus Eupanicum, like other tropical grasses, had high phosphoenolpyruvate carboxylase (E.C. 4.1.1.31) activity, had specialized chloroplasts within the parenchyma bundle sheath cells, and lacked photorespiration. The pattern for the temperate subgenus Dichanthelium was opposite.*

Tropical grasses can fix carbon dioxide at a rate almost twice that of temperate species (*1*). The lower photosynthetic rate of temperate leaves is probably a result of photorespiration, because blocking this process by lowering the ambient oxygen concentration (*2*) can increase the rate of photosynthesis to approximately that of tropical leaves (*3*). Temperate plant yields can also be increased substantially under these conditions (*4*). Consequently genetic selection for greater carbon conservation during photosynthesis as a means of increasing dry matter production among temperate grass crops is particularly attractive. At the same time, the introduction of temperate or tropical characters into species of the opposite type may extend their range of cultivation.

To date we have been unable to test these possibilities because of the lack of variability within a phylogenetic line. That is, genera belonging to the same phylogenetic group have basically the same photosynthetic physiology and internal leaf anatomy. Therefore we have been interested in species of any groups reported to have characteristics that are in apparent disagreement with former correlation data (*5*). The report (*6*) that parenchyma bundle sheath cells of the rosette leaves of *Panicum lindheimeri* do not contain "specialized starch plastids typical of the tribe" was valuable in this regard. This species belongs to the subgenus Dichanthelium, a group of more than 100 species confined chiefly to eastern North America (*7*). Since our previous survey considered only members of the largely tropical subgenus Eupanicum, we have extended it to include some dichantheloid species. It is evident that two different functional patterns exist within this economically important genus.

Photorespiration was estimated by measuring the carbon dioxide compensation point of detached leaves. They were illuminated at 33,000 lu/m^2 in a closed system, and their gas exchange was monitored by an infrared carbon dioxide analyzer. Those leaves that evolved carbon dioxide during photosynthesis reached an equilibrium carbon dioxide concentration (compensation point) of approximately 50 ppm. Species lacking photorespiration compensated at 5 ppm or less (*5*, *8*). To determine initial photosynthetic products, leaves were illuminated at 11,000 lu/m^2 and then exposed to $^{14}CO_2$ in air for 6 seconds; they were then killed and extracted in boiling 80 percent ethanol. Compounds were resolved on paper strips with a liquified phenol, acetic acid, water, 1*M* ethylenediaminetetraacetic acid system (840 : 160 : 10 : 1) (*9*). A chromatogram scanner was used to measure the amount of ^{14}C incorporated into photosynthetic intermediates. Phosphoenolpyruvate (PEP) carboxylase (E.C. 4.1.1.31) activity was assayed according to Slack and Hatch (*10*). Free-hand cross sections of leaves were examined microscopically for anatomical detail. Addition of iodine–potassium iodide to the water mount indicated the areas of starch accumulation.

Members of the subgenus Eupanicum, such as *Panicum bulbosum*, *P. capillare*, and *P. miliaceum*, like other species of the panicoid and chloridoid–