Lake, D. B., and Hoh, G. L. K. (1963), *J. Amer. Oil Chem. Soc. 40*, 628.
Lippert, J. L., and Peticolas, W. L. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1572.
Muesing, R. A., and Nishida, T. (1971), *Biochemistry 10*, 2952.
Oldfield, E., and Chapman, D. (1971), *Biochem. Biophys. Res. Commun. 43*, 610.
Rhodes, D. N., and Lea, C. H. (1957), *Biochem. J. 65*, 527.
Rouser, G., Nelson, G. J., Fleischer, S., and Simon, G. (1968), *in* Biological Membranes: Physical Fact and Function, Chapman, D., Ed., London, Academic, p 5.
Salsbury, N. J., and Chapman, D. (1968), *Biochim. Biophys. Acta 163*, 314.
Sheard, B. (1969), *Nature (London) 223*, 1057.
Shinitzky, M., Dianoux, A.-C., Gitler, C., and Weber, G. (1971), *Biochemistry 10*, 2106.
Small, D. M. (1967), *J. Lipid Res. 8*, 551.
Spencer, R. D., and Weber, G. (1969), *Ann. N. Y. Acad. Sci. 158*, 361.
Veksli, Z., Salsbury, N. J., and Chapman, D. (1969), *Biochim. Biophys. Acta 183*, 434.
Weber, G. (1971), *J. Chem. Phys. 55*, 2399.

# Amino Acid Sequences at Constant and Variable Regions of Heavy Chains of Monotypic Immunoglobulins G and M of a Single Patient†

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ABSTRACT: Previous work indicated that the amino (N)-terminal 34 residues (which includes one hypervariable region) of heavy chains of monotypic immunoglobulins G2-κ and M-κ from a single patient (Til) are identical, and that these two molecules share idiotypic determinants not present in their isolated light chains or in any of a large number of other immunoglobulins tested. Our present data demonstrate that the amino acid sequences of the μ and γ2 chains of this patient are also identical from residues 83 to 108, which includes two other hypervariable regions. These data furnish strong support for the concept that the constant and the variable regions of each immunoglobulin polypeptide chain are synthesized by different structural genes. Examination of amino acid sequences reported for variable regions indicates that tyrosine occurs frequently either within or at the immediate neighborhood of hypervariable regions. Thus, amino acid sequence data of monotypic immunoglobulins support the concept proposed by Singer and his colleagues that tyrosine may play an important role in antigen combining sites. Further amino acid sequence analyses show that the N-terminal 38 residues of the Fcμ fragment of Til IgM are identical with those reported for another IgM, whereas the N-terminal 60 residues of Fcγ2 fragment of Til IgG2 showed approximately 95% amino acid sequence homology at the $C_H2$ domain with the other three γ-chain subclasses. This degree of homology is markedly higher than that of the hinge region, where only 60% homology is observed among the four γ-chain subclasses.

In 1969, we reported on an unusual patient (Til) whose serum had greatly elevated levels of two monotypic proteins: $IgG_2$-κ[1] and IgM-κ (Wang *et al.*, 1969). The light chains of these two monotypic proteins were identical by several criteria, including peptide mapping, electrophoretic mobility in starch gel containing urea at pH 3 and 8, amino acid composition, amino acid sequence of the N-terminal 38 residues, optical rotatory dispersion, and circular dichroism properties (Wang *et al.*, 1969; Pink *et al.*, 1971). Further, the variable regions of the μ and the γ2 chain were identical for their N-terminal 34 residues which include one hypervariable region, and they shared idiotypic determinants not present in their isolated light chains nor in any of a large number of other immunoglobulins tested (Wang *et al.*, 1970b). The significance of these findings upon the two genes–one polypeptide chain hypothesis as well as the switch from IgM to IgG synthesis during the course of an immune response has been discussed extensively in several symposia (Fudenberg *et al.*, 1971; Nisonoff *et al.*, 1972) and a review article (Pink *et al.*, 1971). The present paper presents additional data on the amino acid residues around two other hypervariable regions (residues 83–108) which were defined by Kehoe and Capra (1971), and at the $C_H2$ domain for both the μ and the γ2 chains of this patient (Til). The implications of these sequence data on the genetics and evolution of immunoglobulin molecules are discussed.

## Materials and Methods

*Purification of Proteins.* The monotypic IgG and IgM were isolated by a procedure including sodium sulfate precipitation, ion-exchange chromatography, starch block electrophoresis, and gel filtration on Sephadex columns (Wang *et al.*, 1969).

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[1] The terminology we have used is as far as possible that recommended by the world Health Organization (*W. H. O. Bull. 33*, 721 (1965); *35*, 953 (1966); *38*, 151 (1968); *41*, 975 (1969)).

*Trypsin Digestion of IgM.* The monotypic IgM was subjected to trypsin digestion at high temperature essentially as described by Plaut *et al.* (1972). The IgM was dissolved in a 0.05 M Tris buffer (pH 8.1) in the presence of 0.0115 M calcium chloride at a protein concentration of about 30 mg/ml. This solution was heated to 65° immediately prior to the addition of Tos-PheCH$_2$Cl-treated trypsin (Worthington) at an enzyme : protein ratio of 1 :25 by weight. The digestion was carried out for 7 min and stopped by immersing in ice water at 4°.

*Papain Digestion of IgG$_2$.* The monotypic IgG$_2$ was subjected to papain digestion using the method of Gergeley *et al.* (1967). The IgG$_2$ molecule was dissolved in phosphate buffer (0.075 M, pH 7.0) containing 0.075 M NaCl and 0.002 M EDTA. This solution was incubated at 37° for 6 hr with papain (Worthington, lot no. 61c) at an enzyme : protein ratio of 1 :100 by weight in the presence of 0.01 M 2-mercaptoethanol. The digestion was terminated by the addition of 50% excess of iodoacetamide.

*Affinity Chromatography.* The Sepharose–antibody conjugate was prepared by a modified procedure (Wilchek *et al.*, 1971) of Axen *et al.* (1967). Sepharose 4B was washed on a sintered-glass funnel with water. The washed Sepharose (100 g wet wt) was suspended in water (300 ml) and solid cyanogen bromide (10 g) was added to the suspension. The pH of the solution was brought to 11 with 5 N NaOH and kept between pH 10.8 and 11.2 for 8 min by the addition of NaOH. Continuous stirring during the reaction assured completed dissolution of the cyanogen bromide during the first 5 min. The reaction was terminated by filtration and washing with water. The activated Sepharose was added to the antibody, both of which were in solutions of 0.1 M $NaHCO_3$ (pH 8). The ratio of wet weight of Sepharose to weight of antibody was 30:1. The concentration of protein in the solutions varies from 0.5 to 5 mg per ml in different preparations. The suspension was stirred slowly at 4° for 16 hr and then washed until no more absorbance was detected in the filtrate.

*Cyanogen Bromide Cleavage.* This was carried out in 70% formic acid at room temperature for 4 hr using 2.5 mg of cyanogen bromide for each mg of protein.

*Reduction and Alkylation.* Lyophilized proteins (or peptides) were reduced with 0.01 M dithiothreitol in 7 M guanidine hydrochloride (in 0.5 M Tris buffer, pH 8.4) for 2 hr and alkylated with 0.025 M $^{14}$C-labeled iodoacetic acid.

*NH$_2$-Terminal Sequence Determination.* Amino acid sequence analyses were carried out on an automatic (Beckman Model 890) Protein Sequencer using a procedure (Wang *et al.*, 1971) similar to that of Edman and Begg (1967). About 10 mg of polypeptide chain was dissolved in 0.5 ml of trifluoroacetic acid immediately prior to application to the sequencer. After each cycle of degradation, one residue was cleaved from the N-terminal end of the polypeptide chain in the form of an anilinothiazolinone derivative. This derivative was converted into a phenylthiohydantoin by incubation in 1 N HCl at 80° for 10 min. Occasionally Quadrol was washed over into the fraction collector; in such cases, the mixture of Quadrol and sample was acidified with 0.5 ml of 1 N HCl at room temperature and subsequently extracted with ethyl acetate. Quadrol remained in the aqueous phase under this condition.

The PTH-amino acid residues were identified by gas chromatography on DC-560 or PS-400 columns (Pisano and Bronzert, 1969) before and after silylation and by amino acid analysis of hydrolysates of the PTH-amino acids. The hydrolysis was carried out under reduced pressure in 6 N HCl containing 0.1% phenol for 20 hr at 150° or in 57% HI for 20 hr at 130° (Smithies *et al.*, 1971).

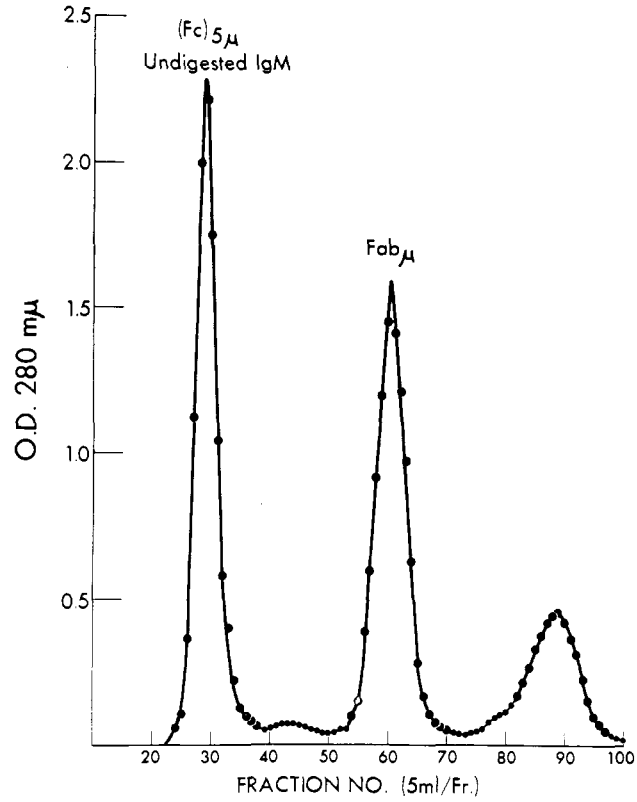


FIGURE 1: The elution profile of the trypsin digest of IgM (Til) on a Sephadex G-200 column (90 cm × 2.5 cm; see text for details) by gel filtration.

## Results

*Fc and Fab Fragments.* The (Fc)5$\mu$ and Fab$\mu$ were prepared by trypsin digestion of monotypic IgM-$\kappa$ isolated from the serum of Til. After digestion, the Fab$\mu$ was isolated by gel filtration on a Sephadex G-200 column (in 0.05 M Tris at pH 8.1). The first peak which came off the column at the void volume contained (Fc)5$\mu$ as well as a small amount of undigested IgM. The second peak contained Fab$\mu$. The first peak, which reacted with anti-$\kappa$ and anti-IgM, was not homogeneous. The second was pure Fab$\mu$, which reacted with anti-$\kappa$ but not with anti-IgM (Figure 1).

The (Fc)5$\mu$ was separated from the undigested IgM by passing the mixture through an immunoadsorbent column in which antibody specific for $\kappa$ light chain was conjugated to Sepharose. The antibody–Sepharose column was washed thoroughly and equilibrated with phosphate-buffered saline prior to the application of the protein mixture to the column. The (Fc)5$\mu$ was eluted under this condition, whereas the undigested IgM was retained because the $\kappa$-chain determinants of Til IgM reacted with the anti-$\kappa$ antibodies which were conjugated with the Sepharose. The undigested IgM was subsequently eluted from the column by 1 M ammonium hydroxide.

The Fc$\gamma$2 and the Fab$\gamma$2 were prepared by papain digestion of monotypic IgG2-$\kappa$ isolated from the serum of Til. They were separated by anion-exchange chromatography on a diethylaminoethyl-cellulose (DE-52, Whatman) column. The column was initially equilibrated with 0.005 M phosphate buffer (pH 8.0) and Fab was eluted under these conditions.

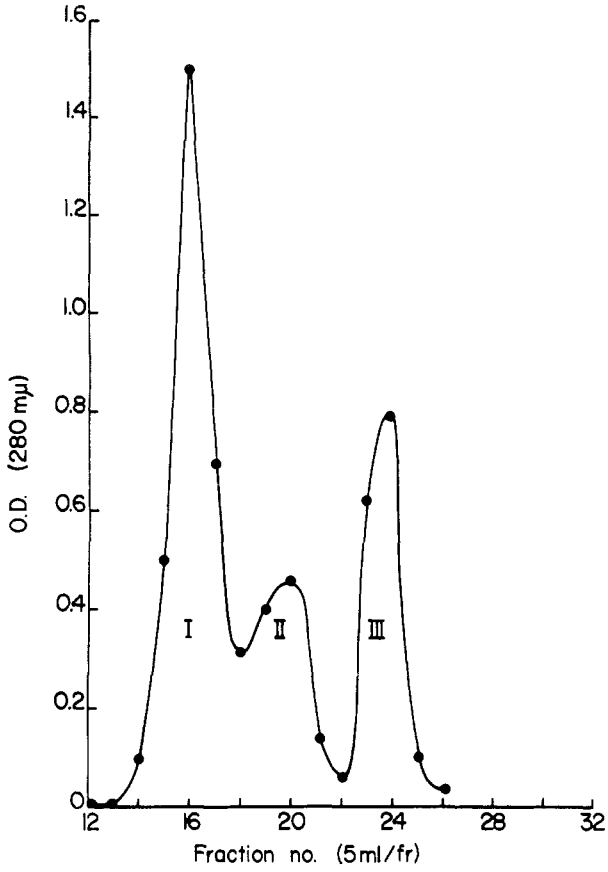


FIGURE 2: The elution profile of peptides obtained from the cyanogen bromide cleavage of the Fcγ2 (Til) on a Sephadex G-50 column (85 cm × 1.7 cm, see text for details) by gel filtration.

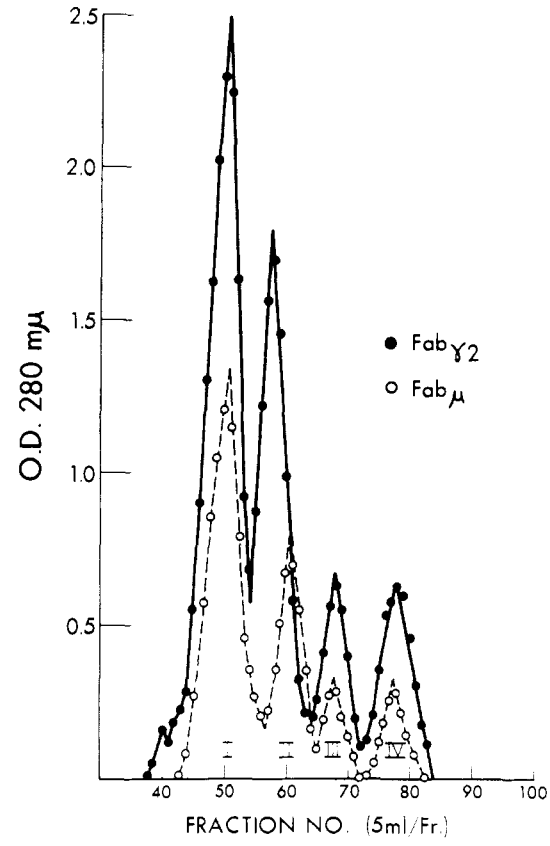


FIGURE 3: The elution profile of peptides obtained from the cyanogen bromide cleavage of Fab fragments on a Sephadex G-100 column (90 cm × 2.5 cm, see text for details) by gel filtration.

TABLE 1: The N-Terminal Residue and Amino Acid Composition of Peptides Subjected to Automatic Sequence Analyses.

| Amino Acid | Percentage[a] of Peptides | | | | |
|---|---|---|---|---|---|
| | A | B | C | Fcγ2 | Fcμ |
| Trp | 1.2 | 1.2 | 1.4 | 0.9 | 1.4 |
| Lys | 8.0 | 7.7 | 5.4 | 10.2 | 3.4 |
| His | 1.8 | 1.5 | 0.2 | 2.8 | 1.8 |
| Arg | 3.9 | 3.1 | 2.5 | 3.3 | 4.4 |
| CM-Cys | 2.3 | 6.1 | 2.5 | 1.5 | 2.6 |
| Asx | 9.6 | 7.7 | 10.2 | 9.6 | 7.4 |
| Thr | 7.3 | 9.3 | 7.1 | 6.4 | 10.2 |
| Ser | 5.5 | 11.1 | 15.5 | 7.2 | 9.3 |
| Glx | 15.6 | 6.7 | 3.6 | 12.0 | 10.9 |
| Pro | 8.5 | 6.9 | 5.0 | 11.6 | 8.0 |
| Gly | 5.7 | 6.6 | 7.6 | 4.9 | 5.4 |
| Ala | 3.9 | 7.2 | 9.0 | 3.0 | 7.3 |
| Val | 10.9 | 10.0 | 9.5 | 10.0 | 8.3 |
| Met | | | | 1.8 | 1.8 |
| Ile | 2.4 | 0.5 | 2.2 | 1.7 | 3.3 |
| Leu | 6.3 | 6.6 | 7.9 | 6.5 | 7.9 |
| Tyr | 3.7 | 4.6 | 6.1 | 3.0 | 2.9 |
| Phe | 3.3 | 3.0 | 4.2 | 3.7 | 3.6 |
| N-Terminal Residue | Ile | Asx | Asx | Ala | Gly |

[a] The total of all amino acids measured was taken as 100%.

The column was then washed with 0.015 M phosphate buffer (pH 8.0) before the elution of the Fcγ2 with 0.5 M phosphate buffer (pH 8.0). The Fcγ2 was homogeneous whereas the Fabγ2 was a mixture of 3.5S monovalent and 5S divalent fragments, as judged by immunoelectrophoresis and ultracentrifugation experiments.

*Cyanogen Bromide Fragments.* The Fcγ2, Fabγ2, and Fabμ were subjected to cleavage by cyanogen bromide. Resultant peptides were completely reduced and alkylated with [$^{14}$C]iodoacetic acid for the labeling of half-cystine residues. Separation of these peptides was done by gel filtration. Figure 2 shows the elution profile of peptides from the cyanogen bromide degradation of the Fcγ2 on a Sephadex G-50 column (1 M acetic acid). Peak I contained a large peptide which was designated as "A" and was subjected to amino acid sequence analysis subsequently. Figure 3 shows the elution profile of peptides obtained from the cyanogen bromide degradation of Fab fragments on a Sephadex G-100 column (1 M acetic acid–6 M urea). Peak I contains intact light chain as judged by immunoelectrophoresis. Peak II from Fabγ2 was designated as "B" and that from Fabμ was designated as "C." Peptide "B" is slightly larger than peptide "C," since "B" came out the column earlier than "C."

*Amino Acid Sequence.* The reduced and alkylated Fcμ, Fcγ2 and peptides "A," "B," and "C" were subjected to amino acid sequence analysis using the automatic protein sequencer. Before applying these peptides to the sequencer, their purity were checked by the dansyl chloride method (Gros and Labonesse, 1969; Wang *et al.*, 1970a) for N-terminal end group analyses. A single dansylamino acid was identified for each of these large peptides by thin-layer chro-

TABLE II: Automatic Edman Degradation of Peptides.

| | Peptide A | | | | Peptide B | | | | Peptide C | | | | Fcγ2 | | | | Fcμ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Identification | | | | Identification | | | | Identification | | | | Identification | | | | Identification | | | |
| Step No. | Amino Acid | Glc | Yield (%)[a] | Residue Assigned | Amino Acid | Glc | Yield (%)[a] | Residue Assigned | Amino Acid | Glc | Yield (%)[a] | Residue Assigned | Amino Acid | Glc | Yield (%)[a] | Residue Assigned | Amino Acid | Glc | Yield (%)[a] | Residue Assigned |
| 1 | Ile | Ile | 52 | Ile | Asx | Asn | 54 | Asn | Asx | Asn | 42 | Asn | Ala | Ala | 37[a] | Ala | Gly | Gly | 44[a] | Gly |
| 2 | Ser | Ser | 41 | Ser | Ser | Ser | 40 | Ser | Ser | Ser | 32 | Ser | Pro | Pro | 49 | Pro | Leu | Leu | 61 | Leu |
| 3 | Arg | | 33 | Arg | Leu | Leu | 38 | Leu | Leu | Leu | 43 | Leu | Pro | Pro | 37 | Pro | Thr | Thr | 50 | Thr |
| 4 | Thr | Thr | 38 | Thr | Arg | | 24 | Arg | Arg | | 30 | Arg | Val | Val | 34 | Val | Phe | Phe | 45 | Phe |
| 5 | Pro | Pro | 44 | Pro | Ala | Ala | 56 | Ala | Ala | Ala | 68 | Ala | Ala | Ala | 34 | Ala | Glx | | 53 | Glx |
| 6 | Glx | Glu | 20 | Glu | Glx | Glu | 26 | Glu | Glx | Glu | 35 | Glu | Gly | Gly | 38 | Gly | Glx | | 42 | Glx |
| 7 | Val | Val | 26 | Val | Asx | Asp | ND | Asp | Asx | Asp | 24 | Asp | Pro | Pro | 45 | Pro | | | | ? |
| 8 | Thr | Thr | 28 | Thr | Thr | Thr | 29 | Thr | Thr | Thr | 24 | Thr | Ser | Ser | 30 | Ser | Ala | Ala | 48 | Ala |
| 9 | | Cys | ND | Cys[b] | Ala | Ala | ND | Ala | Ala | Ala | 48 | Ala | Val | Val | 26 | Val | Ser | Ser | 21 | Ser |
| 10 | Val | Val | 20 | Val | Val | Val | 33 | Val | Val | Val | 28 | Val | Phe | Phe | 24 | Phe | Ser | Ser | 15 | Ser |
| 11 | Val | Val | 38 | Val | Tyr | Tyr | 19 | Tyr | Tyr | Tyr | 19 | Tyr | Leu | Leu | 39 | Leu | | Met | 26 | Met |
| 12 | Val | Val | 44 | Val | Tyr | Tyr | 13 | Tyr | Tyr | Tyr | 14 | Tyr | Phe | Phe | 19 | Phe | | Cys | ND | Cys[b] |
| 13 | Asx | Asp | 19 | Asp | | Cys | ND | Cys[b] | | Cys | ND | Cys[b] | Pro | Pro | 22 | Pro | Val | Val | 34 | Val |
| 14 | Val | Val | 26 | Val | Ala | Ala | 27 | Ala | Ala | Ala | 56 | Ala | Pro | Pro | 25 | Pro | Pro | Pro | 29 | Pro |
| 15 | Ser | Ser | 15 | Ser | Lys | | 20 | Lys | Lys | | 27 | Lys | Lys | | 18 | Lys | Asx | | 21 | Asx |
| 16 | His | | 17 | His | Gly | Gly | 18 | Gly | Gly | Gly | 37 | Gly | Pro | Pro | 20 | Pro | Glx | | 19 | Glx |
| 17 | Glx | | 19 | Glx | Lys | | 18 | Lys | Lys | | 26 | Lys | Lys | | 16 | Lys | Asx | | 13 | Asx |
| 18 | Asx | | 16 | Asx | Val | Val | 11 | Val | Val | Val | 19 | Val | Asx | Asp | 16 | Asp | Thr | Thr | 18 | Thr |
| 19 | Pro | Pro | 25 | Pro | Ser | Ser | 6 | Ser | Ser | Ser | 4 | Ser | Thr | Thr | 18 | Thr | Ala | Ala | 26 | Ala |
| 20 | Glx | | 15 | Glx | Ala | Ala | 20 | Ala | Ala | Ala | 12 | Ala | Leu | Leu | 13 | Leu | Ile | Ile | 15 | Ile |
| 21 | Val | Val | 22 | Val | Tyr | Tyr | 4 | Tyr | Tyr | Tyr | 7 | Tyr | | Met | ND | Met | Arg | | 8 | Arg |
| 22 | Glx | | 12 | Glx | Tyr | Tyr | 4 | Tyr | Tyr | Tyr | 8 | Tyr | Ile | Ile | 13 | Ile | Val | Val | 16 | Val |
| 23 | Phe | Phe | 11 | Phe | Phe | Phe | 7 | Phe | Phe | Phe | 10 | Phe | Ser | Ser | 6 | Ser | Phe | Phe | 9 | Phe |
| 24 | Asx | | 14 | Asx | Asx | | 5 | Asx | Asx | | 7 | Asx | Arg | | 7 | Arg | Ala | Ala | 20 | Ala |
| 25 | | Trp | 20 | Trp | Tyr | Tyr | 3 | Tyr | Tyr | Tyr | 4 | Tyr | Thr | Thr | ND | Thr | Ile | Ile | 16 | Ile |
| 26 | Tyr | Tyr | 10 | Tyr | | | | | | | | | Pro | Pro | 17 | Pro | Pro | Pro | 14 | Pro |
| 27 | Val | Val | 21 | Val | | | | | | | | | Glx | | 10 | Glx | Pro | Pro | 12 | Pro |
| 28 | Asx | | 9 | Asx | | | | | | | | | Val | Val | 10 | Val | Ser | Ser | 3 | Ser |
| 29 | Gly | Gly | 13 | Gly | | | | | | | | | Thr | Thr | ND | Thr | Phe | Phe | 6 | Phe |
| 30 | Val | Val | 18 | Val | | | | | | | | | | Cys | ND | Cys[b] | Ala | Ala | 8 | Ala |
| 31 | Glx | | 6 | Glx | | | | | | | | | Val | Val | 8 | Val | Ser | Ser | 3 | Ser |
| 32 | Val | Val | 12 | Val | | | | | | | | | Val | Val | 6 | Val | Ile | Ile | 7 | Ile |
| 33 | His | | 6 | His | | | | | | | | | Val | Val | 10 | Val | Phe | Phe | 5 | Phe |
| 34 | Asx | | 4 | Asx | | | | | | | | | Asx | | 7 | Asx | Leu | Leu | 6 | Leu |
| 35 | Ala | Ala | 13 | Ala | | | | | | | | | Val | Val | 6 | Val | | | | ? |
| 36 | Lys | | 3 | Lys | | | | | | | | | Ser | Ser | ND | Ser | Lys | | 4 | Lys |
| 37 | Thr | Thr | 4 | Thr | | | | | | | | | His | | 5 | His | Ser | Ser | 2 | Ser |
| 38 | Lys | | 3 | Lys | | | | | | | | | Glx | | 5 | Glx | Thr | Thr | 3 | Thr |
| 39 | Pro | Pro | 3 | Pro | | | | | | | | | Asx | | 5 | Asx | | | | |

[a] Yields of Ser, Thr, Met, and Trp were based on gas chromatography data. Yields of other amino acids were based on amino acid analysis data. Because absolute molecular weights of peptides were not determined, yields are approximate estimates only. [b] The identification of Cys was confirmed by counting the radioactivity of $^{14}C$ using a scintillation spectrometer (Packard, Model 544). ND = not determined; glc = gas chromatography.

TABLE III: Comparison of Amino Acid Sequences of the Variable Region of Til $\gamma 2$ and Til $\mu$ Chains Thus far Determined to Those of Several Other $\gamma 1$ Chains and One $\mu$ Chain.[a]

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                                5                  10                  15                  20                  25
Til  γ2 VHIII Glu-Val-Gln-Leu-Leu-Glu-Ser-Gly-Gly-Gly-Leu-Val-Gln-Pro-Gly-Gly-Ser-Leu-Arg-Leu-Ser-Cys-Ala-Ala-Ser
Til  μ  VHIII ————————————————————————————————————————————————————————————————————————————————————————————
Nie  γ1 VHIII PCA————————Val-Gln————————————Val————————————Arg————————————————————————————————————————
Eu   γ1 VHI   PCA————————Val-Gln————Ala-Glu-Val-Lys-Lys————Ser——Val-Lys-Val————Lys————————————————
Ou   μ  VHII  PCA——Thr——Thr————————Pro-Ala————Lys——Lys-Gln-Pro——Thr——Thr——Thr-Phe————————————
Cor  γ1 VHII  PCA——Thr——Arg————————Pro-Ala————Lys——Thr-Gln-Thr——Thr——Thr——Thr-Phe————————————
Daw  γ1 VHII  PCA——Thr——Arg————————Pro-Ala————Arg——Thr-Gln-Thr——Thr——Thr——Thr-Phe————————————
He   γ1 VHII  PCA——Thr——Lys——Asn——Pro-Thr————Lys——Thr-Glu-Thr——Thr——Thr——Thr-Leu————————————

                     26                  30                  35                  40                  45
Til  γ2 VHIII Gly-Phe-Thr-Phe-Ser-Thr-Tyr-Val-Met
Til  μ  VHIII ————————————————————————————
Nie  γ1 VHIII ————————————————Arg——Thr-Ile- [      ]-His-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Val
Eu   γ1 VHI   ——Gly————————Arg-Ser- Ala-Ile- [      ]-Ile- Trp-Val-Arg-Gln-Ala-Pro-Gly-Gln-Gly-Leu-Glu-Trp-Met
Ou   μ  VHII  ————Ser-Leu————Ser- Arg——Arg-Val-Ser-Trp-Ile- Arg-Arg-Pro-Pro-Gly-Lys-Ala-Leu-Glu-Trp-Leu
Cor  γ1 VHII  ————Ser-Leu——Ser- Thr-Gly——Cys-Val-Gly-Trp-Ile- Arg-Gln-Pro-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Leu
Daw  γ1 VHII  ————Ser-Leu——Gly-Glu-Thr——Cys-Val-Ala-Trp-Ile- Arg-Gln-Pro-Pro-Gly-Glu-Ala-Leu-Glu-Trp-Leu
He   γ1 VHII  ——Leu-Ser-Leu-Thr——Asp-Gly-Val-Ala-Val-Gly-Trp-Ile- Arg-Gln-Gly-Pro-Gly-Arg-Ala-Leu-Glu-Trp-Leu

                     50                  55                  60                  65                  70
Nie  γ1 VHIII Ala-Val-Met-Ser-Tyr-Asx-Gly-Asx-Asx-Lys-His-Tyr-Ala-Asp-Ser-Val-Asn-Gly-Arg-Phe-Thr-Ile-Ser-Arg-Asn
Eu   γ1 VHI   Gly-Gly-Ile-Val-Pro-Met-Phe-Gly-Pro-Pro-Asn-Tyr-Ala-Gln-Lys-Phe-Gln-Gly-Arg-Val-Thr-Ile-Thr-Ala-Asp
Ou   μ  VHII  Ala-Arg-[  ]-Ile-Asx-Asx-Asx-Asn-Lys-Phe-Tyr-Trp-Ser-Thr-Ser-Leu-Arg-Thr-Arg-Leu-Ser-Ile-Ser-Lys-Asn
Cor  γ1 VHII  Ala-Arg-[  ]-Ile-Asx-Trp-Asp-Asp-Asp-Lys-Tyr-Tyr-Asx-Thr-Ser-Leu-Glx-Thr-Arg-Leu-Thr-Ile-Ser-Lys-Asp
Daw  γ1 VHII  Ala-Trp-Asp-Ile-Leu-Asn-Asp-Asp-[  ]-Lys-Tyr-Tyr-Gly-Ala-Ser-Leu-Glu-Thr-Arg-Leu-Ala-Val-Ser-Lys-Asp
He   γ1 VHII  Ala-Trp-Leu-Leu-Tyr-Trp-Asp-Asp-Asp-Lys-Arg-Phe-Ser-Pro-Ser-Leu-Lys-Ser-Arg-Leu-Thr-Val-Thr-Arg-Asp

                     75                  80                  85                  90                  95
Til  γ2 VHIII                                    Met-Asn-Ser-Leu-Arg-Ala-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Lys
Til  μ  VHIII                                    ————————————————————————————————————————————————————————
Nie  γ1 VHIII Asp-Ser-Lys-Asn-Thr-Leu-Tyr-Leu-Asn————————————Pro-Glx-Asx————————————————————————Arg
Eu   γ1 VHI   Glu-Ser-Thr-Asn-Thr-Ala-Tyr-Met-Glu-Leu-Ser————————Ser————————Phe——Phe————Gly
Ou   μ  VHII  Asp-Ser-Lys-Asn-Gln-Val-Val-Leu-Ile————Ile-Asn-Val-Asn-Pro-Val————Thr————Arg
Cor  γ1 VHII  Thr-Ser-Arg-Asn-Gln-Val-Val-Leu-Thr——Asp-Pro-Val-[      ]————Thr————Arg
Daw  γ1 VHII  Thr-Ser-Lys-Asn-Gln-Val-Val-Leu-Ser————Thr-Val-Gly-Pro-Gly————Thr————Arg
He   γ1 VHII  Thr-Ser-Lys-Asn-Gln-Val-Val-Leu-Thr——Thr-Asn-Met-Asp-Pro-Val————Thr————Val-His

                    100                 105                 110
Til  γ2 VHIII Gly-Lys-Val-Ser-Ala-Tyr-Tyr-Phe-Asx-Tyr
Til  μ  VHIII ——————————————————————————
Nie  γ1 VHIII Ile-Arg-Asp-Thr——Met-Phe——Ala-His [      ]-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val
Eu   γ1 VHI   ——Tyr-Gly-Ile-Tyr-Ser-Pro-Glu-Glu——[      ]-Asn-Gly-[  ]-Gly-[  ]-Leu-Val-Thr-Val
Ou   μ  VHII  Val-Val-Asn——Val-Met-Ala-Gly-Tyr——Tyr-Tyr-Met-Asp-Val-Trp-Gly-Lys-Gly-Thr-Thr-Val-Thr-Val
Cor  γ1 VHII  Ile-Thr——Ile-Pro-Ala-Pro-Ala-Gly——[      ]-Met-Asp-Val-Trp-Gly-Arg-Gly-Thr-Pro-Val-Thr-Val
Daw  γ1 VHII  Ser-Cys-Gly——Gln-[  ]————Asp——[      ]-Trp-Gly-Gln-Gly-Ile-Leu-Val-Thr-Val
He   γ1 VHII  Arg-His-Pro-Arg-Thr-Leu-Ala——Asp-Val-[      ]-Trp-Gly-Gln-Gly-Thr-Lys-Val-Ala-Val
```

[a] Residues are numbered after the sequence of Eu. References for the sequences are: Til $\gamma 2$ and $\mu$ (Wang *et al.*, 1971, and this paper), Nie (Ponstingl *et al.*, 1970), Eu (Edelman *et al.*, 1969), Ou (Shimizu *et al.*, 1971), Cor and Daw (Press and Hogg, 1969), and He (Cunningham *et al.*, 1969). Solid lines indicate identity to the seqence of Til $\gamma 2$ on the top line. [] were introduced to assure maximum homology.

matography on silica gel. Table I shows the results of the N-terminal residue determination and amino acid composition of these peptides. Results of the amino acid sequence analyses are shown in Table II. The amino acid sequences for the N-terminal 38 residues of the Fc$\mu$ (Til) is identical with that of another IgM protein (Ou) reported by Shimizu *et al.* (1971). Comparison of this sequence with that of Fc of $\gamma$ chains showed less than 15% homology irrespective which part of the $\gamma$ chain was compared. The N-terminal sequence of peptide "A" overlaps that of Fc$\gamma 2$. The combined data have established the amino acid sequence for the N-terminal 60 residues of Fc$\gamma 2$ fragment.

The N-terminal 25 residues of peptide "B" are identical with that of peptide "C." Examination of variable-region amino acid sequences of other human immunoglobulins indicate that these peptides start at position 84 on the heavy

TABLE IV: Comparison of Amino Acid Sequences of the Constant Region of Til γ2 Chains Thus far Determined to Those of a γ1 Chain (Eu, Edelman *et al.*, 1969) a γ4 chain (Vin, Milstein and Pink, 1970) and a γ3 chain (Kup, Frangione and Milstein, 1968).[a]

| | 231 | 235 | 240 | 245 | 250 | 255 |
|---|---|---|---|---|---|---|
| γ2 (Til) | Ala-Pro-Pro-Val-Ala[GAP]-Gly-Pro-Ser-Val-Phe-Leu-Phe-Pro-Pro-Lys-Pro-Lys-Asp-Thr-Leu-Met-Ile-Ser-Arg- | | | | | |
| γ1 (Eu) | ————Glu-Leu-Leu-Gly———— | | | | | |
| γ4 (Vin) | ————Ser——Phe———— | | | | | |
| γ3 (Kup) | ————Glu-Leu | | | | ———(————)———— | |

| | 256 | 260 | 265 | 270 | 275 | 280 |
|---|---|---|---|---|---|---|
| γ2 (Til) | Thr-Pro-Glu-Val-Thr-Cys-Val-Val-Val-Asp-Val-Ser-His-Glx-Asx-Pro-Glx-Val-Glx-Phe-Asx-Trp-Tyr-Val-Asx | | | | | |
| γ1 (Eu) | ———————————————————————Glu-Asp——Gln——Lys——Asn————Asp | | | | | |
| γ4 (Vin) | ———————————————————Gln-Glu-Asp———————————————— | | | | | |
| γ3 (Kup) | ——————————(————————) | | | | | |

| | 281 | 285 | 290 291 |
|---|---|---|---|
| γ2 (Til) | Gly-Val-Glx-Val-His-Asx-Ala-Lys-Thr-Lys-Pro | | |
| γ1 (Eu) | ————Gln————Asn———————— | | |
| γ4 (Vin) | ————Glu————Asn———————— | | |

[a] Residues are numbered after the sequence of Eu. Sequences in parentheses are not firmly established. Solid lines indicate identity to the sequence of γ2 (Til) on the top.

chains resulting from the cleavage of a peptide bond in which methionine (at position 83) contributed the carboxyl group. The exact sizes of these peptides were not determined but estimations based on their elution profile from a calibrated Sephadex G-100 column indicates they each contain approximately 110–140 amino acids. Thus, peptides "B" and "C" represent the C-terminal portion of the VH domain and the major portion of the $C_H1$ domain or the γ2 and the μ chain, respectively.

## Discussion

In previous work we have demonstrated that the N-terminal 34 residues of the μ and the γ2 chain isolated from Til are identical (Wang *et al.*, 1971). The present study showed that these two heavy chains are identical also for the 26 residues from 83 to 108.

Table III compares the available amino acid sequence data from the variable region of Til μ and γ2 chains with published data on six other heavy chains. The Til γ2 and Til μ chains are identical and show 70% homology with another VHIIIγ1 chain (Nie, 42 of 60 residues), compared with 35–48% homology (21–29 of 60 residues) with heavy chains of VHI and VHII subgroups. (These percentages of homology should not be taken as representative of inter- and intra-subgroup homology, since nearly 30 of the residues involved are located at hypervariable regions).

Based on amino acid sequence data on the variable region, four hypervariable regions have thus far been described for heavy chains (Kabat and Wu, 1971; Kehoe and Capra, 1971; Wang *et al.*, 1971). They comprise residues 31–35, 50–65, 81–89, and 98–107. These hypervariable regions correlated well with the antigen combining site as judged by affinity-labeling experiments (Singer *et al.*, 1971; Haimovich *et al.*, 1972). It is striking that the amino acid sequence of Til γ2 chain and Til μ chain are identical at three of the four hypervariable regions thus far sequenced (Table III). Therefore, our findings in Til μ and γ2 heavy chains furnish strong support for the concept that the variable region and the constant region of each immunoglobulin polypeptide chain are synthesized by different structural genes (Dreyer and Bennett, 1965).

Our data also strengthen our earlier proposal (Wang *et al.*, 1970b) on the genetic switching mechanism whereby a given variable-region gene can be translocated (Gally and Edelman, 1970) to the constant-region gene of the μ chain to signal the synthesis of IgM antibody at the early stage of an immune response and later switched to the constant region gene of a γ chain to initiate the synthesis of IgG antibody. Presumably this translocation event is closely related to the phenomena of allelic exclusion and specific gene activation and committing the cell to the production of a specific antibody. Other examples of multiple myeloma have since been reported in which shared idiotypic determinants were observed for IgG and IgM (Penn *et al.*, 1970) as well as for IgG and IgA (Rudders *et al.*, 1972). These observations suggest that the genetic switch mechanism also involves α chains. It is likely that switching of variable- and constant-region genes is a common phenomenon in the cytodifferentiation of immunocytes (Wang *et al.*, 1972).

It is interesting to note that tyrosine is frequently observed either within or at the immediate neighborhood of the hypervariable region, for example, at positions 108, 95, 94, 80, 60, and 32 of heavy chains (Table III). A quantitative examination based on reported amino acid sequence data on heavy-chain variable regions (summarized in Table III) indicated that tyrosine occurred 43 times among the 389 residues which cover positions 80–110, 50–65, and 30–35, whereas it did not occur at any of the other positions which included 444 residues listed in Table III. A similar situation was also observed in the amino acid sequences of light chains in which tyrosine occurs with high frequency at positions in or near hypervariable regions, namely, positions 32, 36, 49, 86, 87, and 91 (Wu and Kabat, 1970), but very sparsely at any other position on the entire variable region. (Hypervariable regions of light chains comprise residues 27–34, 50–56, and 90–97, Wu and Kabat, 1970.) This difference is more than coincidental and suggests that tyrosine may play a role in the antigen combining site.

Singer and his colleagues (Singer *et al.*, 1971; Singer and Doolittle, 1966) have demonstrated that tyrosine residues were most frequently labeled in their affinity-labeling experiment at the active sites of a wide range of antibody specificity. However, as the affinity-labeling reagents (*p*- and *m*-nitrobenzenediazonium fluoborate, etc.) they used reacted preferentially with tyrosine, it is difficult to ascertain the significance of their result. Our present analysis furnishes additional support for the importance of tyrosine residues in connection with the antigen combining sites.

Table IV compares the first 60 N-terminal amino acid residues of Fc$\gamma$2 (Til) to the homologous region of a $\gamma$1 chain Eu (Edelman *et al.*, 1969) and a $\gamma$4 chain Vin (Milstein and Pink, 1970). It is noteworthy that the amino acid sequences of these polypeptide chains are very similar after residue 237. Of the 55 residues between 236 and 292, only three positions differed among the four $\gamma$-chain subclasses, constituting about 95% homology. At position 268, His was found in $\gamma$1 and $\gamma$2 and Gln in $\gamma$4; at position 274, Glx was found in $\gamma$2 and $\gamma$4 and Lys in $\gamma$1; and at position 283, Gln was found in $\gamma$1 and Glu in $\gamma$4. The sequence of $\gamma$3 chain at this area has not been completely determined, although preliminary data shows that it is very similar to those of the other $\gamma$-chain subclasses (Frangione and Milstein, 1968). This is quite different from the hinge region, covering about 30 residues immediately before position 237, where only 60% sequence homology was observed among the four $\gamma$-chain subclasses (de Preval *et al.*, 1970). These differences in homology for different parts of the constant region indicate that the hinge region probably has evolved at a much faster rate than the rest of the $\gamma$-chain constant region. However, in view of the wealth of the hinge region in proline, or its vicinity to inter-heavy-chain disulfide bonds, other possibilities certainly exist. One example of speculation is that the hinge region may have been inserted into the constant region like an episome at the gene level.

The N-terminal sequence of the Fc$\gamma$2 (Til) also suggests that papain splits the $\gamma$2 chain initially on the carboxyl side of the inter-heavy-chain disulfide bridges whereas previous reports have claimed that papain splits IgG molecules on the amino side of the inter-heavy-chain disulfide bridges (Porter, 1959; Hsiao and Putnam, 1961; Heimer *et al.*, 1965; Utsumi and Karush, 1965). Detailed study of papain digestion on human $IgG_2$ myeloma proteins and its implications have been reported elsewhere (Wang and Fudenberg, 1972).

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## References

Axen, R., Porath, J., and Ernback, S. (1967), *Nature (London) 214*, 1302.

Cunningham, B. A., Pflumm, M. N., Rutishauser, V., and Edelman, G. M. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 997.

de Preval, C., Pink, J. R. L., and Milstein, C. (1970), *Nature (London) 228*, 930.

Dreyer, W. J., and Bennett, J. C. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 864.

Edelman, G. M., Cunningham, W. E., Gottlieb, P. D., Rutishauser, V., and Waxdal, M. J. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 78.

Edman, P., and Begg, G. (1967), *Eur. J. Biochem. 1*, 80.

Frangione, B., and Milstein, C. (1968), *J. Mol. Biol. 33*, 893.

Fudenberg, H. H., Wang, A. C., Pink, J. R. L., and Levin, A. S. (1971), *Ann. N. Y. Acad. Sci. 190*, 501.

Gally, J. A., and Edelman, G. E. (1970), *Nature (London) 227*, 341.

Gergely, J., Medgyesi, G. A., and Stanworth, D. R. (1967), *Immunochemistry 4*, 369.

Gros, C., and Labonesse, B. (1969), *Eur. J. Biochem. 7*, 463.

Haimovich, J., Eisen, H. N., Hurwitz, E., and Givol, D. (1972), *Biochemistry 11*, 2389.

Heimer, R., Schnoll, S. S., and Primack, A. (1967), *Biochemistry 6*, 127.

Hsiao, S. H., and Putnam, F. W. (1961), *J. Biol. Chem. 236*, 122.

Kabat, E. A., and Wu, T. T. (1971), *Ann. N. Y. Acad. Sci. 190*, 382.

Kehoe, J. M., and Capra, J. D. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2019.

Milstein, C., and Pink, J. R. L. (1970), *Progr. Biophys. Mol. Biol. 21*, 209.

Nisonoff, A., Fudenberg, H. H., Wilson, S. K., Hopper, J. E., and Wang, A. C. (1972), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 31*, 206.

Penn, G. M., Kunkel, H. G., and Grey, H. M. (1970), *Proc. Soc. Exp. Biol. Med. 135*, 660.

Pink, J. R. L., Wang, A. C., and Fudenberg, H. H. (1971), *Annu. Rev. Med. 22*, 145.

Pisano, J. J., and Bronzert, T. J. (1969), *J. Biol. Chem. 244*, 5597.

Plaut, A. G., Calvanico, N., and Tomasi, T. B. (1972), *J. Immunol. 108*, 289.

Ponstingl, H., Schwarz, J., Reichel, W., and Hilschmann, N. (1970), *Hoppe-Seyler's Z. Physiol. Chem. 351*, 1591.

Porter, R. R. (1959), *Biochem. J. 73*, 119.

Press, E. M., and Hogg, N. M. (1969), *Nature (London) 223*, 807.

Rudders, R. A., Yakulis, V., and Heller, P. (1972), *Clin. Res. 20, No. 3*, 499 Abstr.

Shimizu, A., Paul, C., Kohler, H., Shinoda, T., and Putnam, F. W. (1971), *Science 173*, 629.

Singer, S. J., and Doolittle, R. F. (1966), *Science 153*, 13.

Singer, S. J., Martin, N., and Thorpe, N. O. (1971), *Ann. N. Y. Acad. Sci. 190*, 342.

Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G., and Ballantyne, D. L. (1971), *Biochemistry 10*, 4912.

Utsumi, S., and Karush, F. (1965), *Biochemistry 4*, 1766.

Wang, A. C., and Fudenberg, H. H. (1972), *Nature (London), New Biol. 240*, 24.

Wang, A. C., Fudenberg, H. H., Goldrosen, M. H., and Freedman, M. H. (1972), *Immunochemistry 9*, 473.

Wang, A. C., Fudenberg, H. H., and Pink, J. R. L. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1143.

Wang, A. C., Pink, J. R. L., Fudenberg, H. H., and Ohms, J. (1970a), *Proc. Nat. Acad. Sci. U. S. 66*, 657.

Wang, A. C., Wang, I. Y. F., McCormick, and Fudenberg, H. H. (1969), *Immunochemistry 6*, 451.

Wang, A. C., Wilson, S. K., Hopper, J. E., Fudenberg, H. H., and Nisonoff, A. (1970b), *Proc. Nat. Acad. Sci. U. S. 66*, 337.

Wilchek, M., Bocchini, V., Becker, M., and Givol, D. (1971), *Biochemistry 10*, 2828.

Wu, T. T., and Kabat, E. A. (1970), *J. Exp. Med. 132*, 211.