(1957)]; this uraninite gives concordant uranium-lead ages.
13. A duplicate measurement of a solution of sample B by two of us (T.L.K. and M.A.K.) showed complete agreement (*19*). The paired measurements shown in Table 1 are not "duplicates" in the strict sense because the analyzed material of a sample consisted of coral fragments that were not completely homogenized. Thus the data, although showing reproducible age estimates, indicate some inhomogeneities in the uranium distribution in the coral.
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18. If one assumes that these corals initially have $^{234}U/^{238}U$ activity ratios close to those of their living counterparts and the present seawater, that is, 1.14 ± 0.01 [see (*9*); see also M. Koide and E. D. Goldberg, *Progr. Oceanogr.* **3**, 173 (1965) and Table 1], the observed $^{234}U/^{238}U$ values of 1.10 ± 0.015 correspond to an age of $120{,}000 \pm 40{,}000$ years.
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29. Sample locations may be described as follows: samples C1 through C5: conglomerate of basalt and massive coral boulders in a matrix of semilithified bioclastic sand; samples taken from the east side of the head of a gully leading down to the beach, southwest of the end of Black Point Road (samples C1 to C4 were collected by Dr. J. Resig of the University of Hawaii); sample C6: semilithified beach sand and conglomerate with abundant mollusks and coral cobbles; sample taken from the west side of the Kaalakei Street–Kawaihae Street intersection; sample C7: unlithified beach sand with basalt boulders, coral cobbles, and abundant mollusks, above Koolau volcanics and overlain to the north by unlithified aeolianite up to 15 m above sea level; sample taken on the east side of the Kaalakei Street–Kawaihae Street intersection; sample C8: lithified limestone with abundant mollusks and corals; sample taken north of the roadcut (before present widening) opposite 5494 Kalanianaole Highway on the south end of Hawaiiloa Ridge; sample C9: conglomerate with coral and *Strombus* shells in a semilithified sand matrix; 0 to 4 m above sea level; sample taken about 300 m west of the end of paved Farrington Highway; sample C10: conglomerate 1 to 2.5 m thick with bedded beach sand and abundant waterworn corals and mollusks; base of the deposits at 7.5 to 9 m above sea level and unconformably lying upon clay, silt, and tuffaceous sediments; sample found about 300 m east-northeast of Building 1584 in the Kaneohe Marine Corps Firing Range on the east side of Ulupau Head [photo and cross section of the locality presented by H. T. Stearns and K. N. Vaksvik, *Territ. Hawaii Div. Hydrogr. Bull.* **1** (1935), plate 17A, p. 89; figure 10, p. 122]; sample C11: same locality as sample C10, on top of a platform formed by the conglomerate deposits; sample C12: reef limestone cropping out of recent beach sand; sample taken about 1.8 km east of Kakuku Point north of the RCA radio station [see figure 8 in (*16*)]; sample C13: beach conglomerate cemented in a solution cavity in a coral reef of Waimanalo age at the northeastern end of the sand beach at the abandoned concrete Mokapu Landing on Mokapu Point, Ulupau Head (samples supplied by H. T. Stearns); sample C14: well-indurated reef limestone with oyster shells and basalt boulders, overlain unconformably (?) by a hard limestone conglomerate 15 to 30 cm thick from which sample C15 was taken, this in turn overlain by 15 to 30 cm of red soil and 1 to 5 m of black ash; sample taken about 300 m east of the southern end of Kulamanu Place at Black Point [see (*16*), p. 61, and figure V-5 in (*2*)]; sample C16: semilithified conglomerate with cobbles and boulders of coral and shell fragments in coarse calcareous matrix, in two pockets on Diamond Head tuff and overlain by Leahi aeolianite; sample taken on the cliff face 165 m east of Diamond Head lighthouse [see figure 7 in (*16*) for photo of the locality]; sample C17: well-indurated limestone with sparse coral cobbles cropping out in a patch surrounded by Diamond Head tuff at about mean tide level; sample found 120 m east of the end of Diamond Head Road; sample C18: coral embedded in calcareous soil of the Kawela soil of Stearns on pitted surface of reef limestone from which sample C12 was taken; sample taken about 8 m west of the site of sample C12.
30. Research supported by the Earth Science Section, National Science Foundation, under NSF grant GA-36182. We are grateful to Drs. H. T. Stearns and J. Resig for supplying samples. We also thank Dr. Stearns and D. Lum who guided T.L.K. on a field trip. Contribution No. 331 from the Department of Geological Sciences, University of Southern California.

9 October 1973

Immunoglobulin Structure: Amino Terminal Sequences of Mouse Myeloma Proteins That Bind Phosphorylcholine

Abstract. *The amino terminal sequences of five light and heavy immunoglobulin chains from myeloma proteins of the BALB/c mouse with binding activity to phosphorylcholine are presented. Except for a single substitution in position 4, all five heavy chains have identical amino terminal sequences through the first hypervariable region. Proteins which share unique (idiotypic) antigenic determinants are identical through the first hypervariable region of their light and heavy chains. Proteins with differing idiotypic determinants have light chains of differing amino acid sequence. These observations suggest that the heavy chain plays a more important role than the light chain in determining the phosphorylcholine binding site.*

For the past several years, myeloma immunoglobulins produced by plasmacytomas in the BALB/c mouse have been screened against a limited series of antigens (*1*). Approximately 5 percent of these proteins have been shown to exhibit specific binding activity. In many instances it has been possible to assign this binding activity to chemically defined haptens. Indeed, groups of proteins have emerged which bind the same haptenic determinant, for

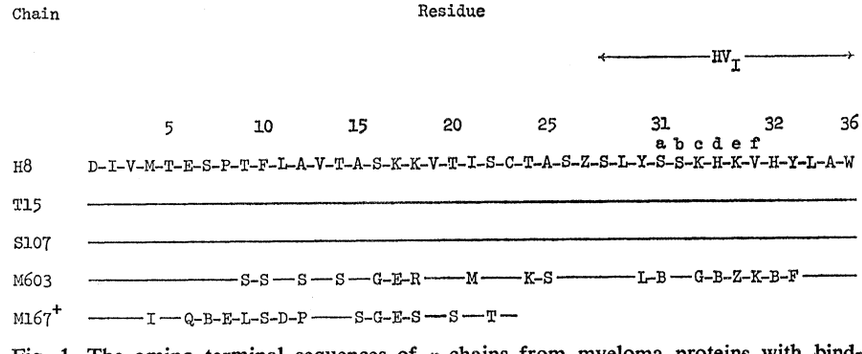

Fig. 1. The amino terminal sequences of κ chains from myeloma proteins with binding activity to phosphorylcholine. HV$_I$ indicates the extent of the first hypervariable region; H8 indicates HOPC 8; T15 indicates TEPC 15; M603 indicates MOPC 603; and M167 indicates MOPC 167 [see (*14*)]. The one-letter amino acid code is: glycine, G; alanine, A; valine, V; leucine, L; isoleucine, I; serine, S; threonine, T; proline, P; cysteine, C; methionine, M; histidine, H; lysine, K; arginine, R; aspartic acid, D; glutamic acid, E; asparagine, N; glutamine, Q; aspartic acid or asparagine, B; glutamine or glutamic acid, Z; tyrosine, Y; phenylalanine, F; tryptophan, W. The numbering of residues for these chains is that taken from a homologous mouse κ chain, MOPC 41 (*19*).

example, dinitrophenol (*2*), phosphorylcholine (*3–5*), α-(1 → 3)dextran (*6*), β-(1 → 6)-D-galactan (*7*), α-(1 → 6)dextran (*8*), β-(2 → 1)fructosan (*8*), and β-(2→ 6) fructosan (*8*). Studies of the primary structure of proteins that bind the same hapten will be useful for delineating structure-function relationships as well as providing possible insights into the genetic mechanism of antibody diversity.

In this study we compare the partial amino acid sequences of light (L) and heavy (H) chains from five BALB/c myeloma proteins that bind phosphorylcholine. Previous studies have indicated that three of the five phosphorylcholine-binding proteins, H8, T15, and S107, bind identical groups of related antigens (*9*). The other two proteins, M603 and M167, bind groups of related antigens which distinguish them from each other and from those of the first group. In addition, immunologic studies have demonstrated that H8, T15, and S107 possess the same individual antigenic specificity (idiotype) suggesting a high degree of structural identity (*5*). [These proteins are members of the S63-T15 idiotypic group (*10*).] In contrast, the other two proteins, M603 and M167, have unique antigenic determinants, which differentiate them from each other and from the group with the shared idiotype (*5*). We were interested in examining the variable regions of the light (V_L) and heavy (V_H) chains from these proteins in order to determine how their amino acid sequences correlated with the antigen binding and idiotypic properties.

The amino acid sequence analyses of partially reduced and alkylated immunoglobulin chains were carried out on a Beckman model 890A or 890C sequencer with the use of standard buffers. The phenylthiohydantoin amino acid derivatives were analyzed by gas chromatography, by thin-layer chromatography, and by amino acid analysis after hydrolysis of the derivatives to free amino acids (*11*). At least two sequenator runs were carried out on each polypeptide chain.

The partial amino acid sequences for L chains from phosphorylcholine-binding immunoglobulins H8, T15, S107, M167, and M603 are given in Fig. 1. Each of these L chains is of the kappa (κ) type. Several important points can be derived from an analysis of these L chain data. It should be noted that L chains have three regions, residues 28 to 34, 50 to 56, and 90 to

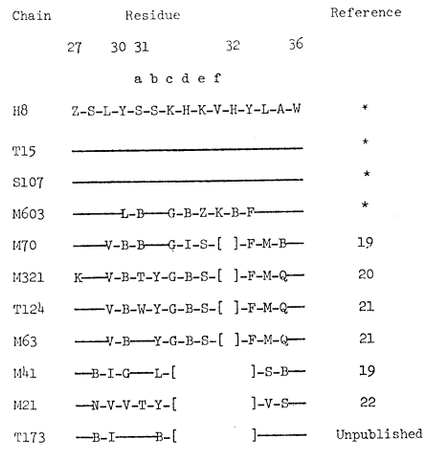

Fig. 2. The first hypervariable region from myeloma κ chains of the BALB/c mouse. The asterisk indicates the data presented in this report.

97, which are far more variable than the remainder of the V region (*12*). These are termed hypervariable regions. Indeed, these regions are so variable that two randomly chosen myeloma L chains, from man or mouse, have a very low probability of showing identity in any one of the hypervariable regions (*13*). Thus it is striking that the L chains from the three proteins demonstrating idiotypic identity, H8, T15, and S107, are identical for their first 41 residues which include the first hypervariable region (Figs. 1 and 2). The M603 L chain differs from the H8 group by 18 out of 41 residues, 8 of which are in the first hypervariable region. The M167 L chain differs from the H8 group at 14 out of the first 23 residues, none of which are in the first hypervariable region (*14*). Thus at least three L chain sequences are present among the five immunoglobulins with phosphorylcholine-binding activity. The first hypervariable region of the four L chains examined is two residues longer than that of any of the mouse κ chains previously reported (Fig. 2). This may reflect a common structural requirement for phosphorylcholine-binding L chains.

The partial amino acid sequences for H chains from phosphorylcholine-binding immunoglobulins H8, T15, S107, M603, and M167 are given in Fig. 3. The amino terminal sequences from these H chains are identical, apart from a single base substitution, leucine to valine, at position 4 in M167. Heavy chains also have hypervariable regions whose properties are similar to those described for L chains. The first hypervariable region of H chains appears to extend from positions 27 to 35 as indicated in Fig. 3. Perhaps the most striking finding in this study is the identity of all five H chains in the first hypervariable region, even though they were derived proteins with three different idiotypes. This observation assumes added importance in that the hypervariable regions appear to be in or near the antigen combining site as judged by affinity label and x-ray crystallographic studies (*15*). Thus these regions may play a particularly critical role in determining the three-dimensional configuration of the combining site. Indeed, the H chain sequence characteristic of the phosphorylcholine-binding myelomas has not been seen in any of the 23 other BALB/c V_H sequences that were derived from immunoglobulins lacking this activity analyzed to date (*16*).

This is, to our knowledge, the first report in which hypervariable regions from both the L and H chains of independently arising immunoglobulins with idiotypic identity have been shown, respectively, to be identical. Thus the results of this partial amino acid sequence analysis and some other studies on the remainder of the V regions are consistent with the supposition that immunoglobulins with identical idiotypic specificities are identical in the

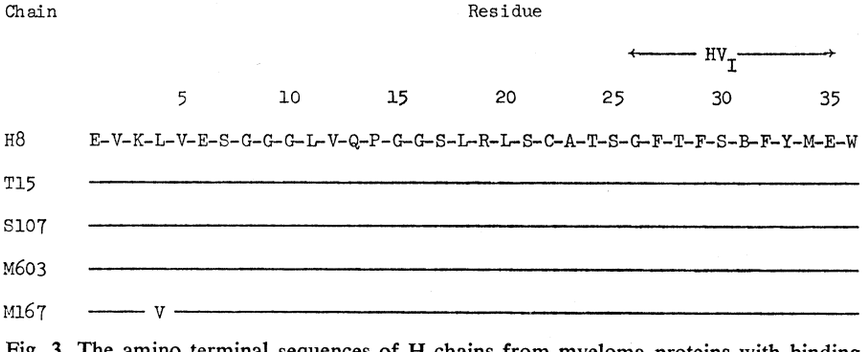

Fig. 3. The amino terminal sequences of H chains from myeloma proteins with binding activity to phosphorylcholine. HV_I indicates the span of the first hypervariable region.

V region sequences of their L and H chains.

If identical idiotypic specificities indicate structural identity, then there are two indications that immunoglobulins of the S63 idiotype are present in normal BALB/c mice. First, immunization of normal BALB/c mice against the phosphorylcholine determinant yields immunocytes producing specific antibody that can be inhibited in the Jerne plaque assay by idiotypic antiserum of the S63 type (*17*). Second, immunoglobulins of the S63 idiotype are found in the normal serum of BALB/c mice (*17a*). These results suggest that the myeloma proteins with phosphorylcholine-binding activity are indeed an excellent model system for studying the relation between structure and function in bona fide antibody molecules.

Since the H chains, even from those proteins with differing idiotypic specificity, are identical for 36 residues, except for a single amino acid substitution, it will be particularly interesting to determine whether the entire H chains from proteins of differing idiotypic specificity are identical (for example, T15 and M603). If so, perhaps this H chain sequence, or one very closely related to it, is a prerequisite for all immunoglobulins with phosphorylcholine-binding activity. It has been shown that all myeloma proteins with α-(1 → 3)dextran activity have an identical lambda L chain (*18*). Proteins with β-(1 → 6)-D-galactan binding activity have very similar sequences in both L and H chains (*7*). Thus in some instances either L or H chain may play a dominant role in hapten binding, while in others a specific L and H pair may be rigidly required.

If immunoglobulins of the S63 idiotype are identical, this suggests that the corresponding V_L and V_H regions are coded by genes in the germ line of the BALB/c mouse, for it is unlikely that a random somatic recombination or somatic mutation process would generate multiple identical antibodies in different individuals. In this regard, it will be interesting to determine whether the H chain from M167 is identical to the others apart from a single substitution outside the hypervariable region, or whether other differences will be found. If a single substitution is present, it could be explained by a single base substitution, either in the germ line or in the soma.

The complete analysis of these proteins as well as those from groups with binding activity to other haptens should continue to provide insights into the structure, genetics, regulation, and evolution of immunoglobulin molecules.

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16 November 1973

Choline Content of Rat Brain

Abstract. *The free choline concentration in the rat brain was found to be 26.3 nanomoles per gram of brain tissue. This value was obtained through use of 6-second microwave irradiation for killing animals and inactivating enzymes, followed by a pyrolysis–gas chromatographic assay procedure. The identities of compounds measured from brain samples were verified by mass spectrometry.*

Knowledge of the concentration of free choline in the brain is important in studies of both the central cholinergic system and phospholipid metabolism. Controversies presently exist concerning the sources of free choline in the brain, for example, the contributions made by choline in the blood and the role of choline produced by the breakdown of brain phospholipids (*1*). Choline is considered a precursor for the synthesis of acetylcholine (*2*). Accurate assessment of acetylcholine turnover through the use of labeled choline depends on knowledge of the size of the endogenous choline pool (*3*). Choline is also a product of acetylcholine metabolism, and changes in the rate of acetylcholine release have been assessed by measuring choline in the cerebrospinal fluid (*4*).

The free choline concentrations reported recently vary widely (*5*). The values for rat brain range from 700 nmole per gram of brain tissue (*6*) down to 170 (*7*), 86.4 (*8*), and 39 nmole/g (*9*). While these values reflect both postmortem changes and differing analytical techniques (*10*), it has been speculated that the concentration of free choline in the brain is even lower than currently being reported. A