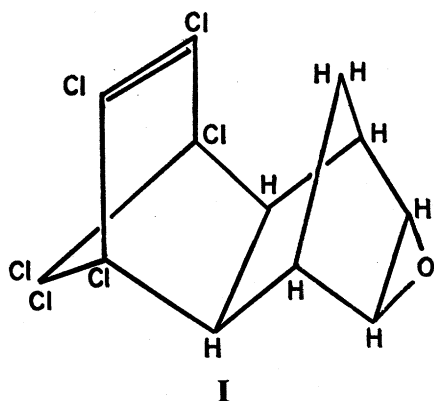


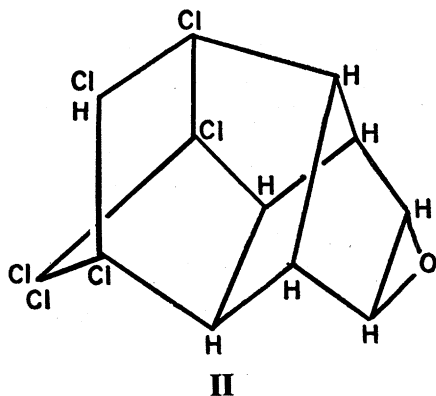


liter of medium) was added to samples of the medium and incubated for 30 days to avoid the possibility of contaminants being confused as the metabolic products by microbes. Under such treatment neither photodieldrin nor any other metabolites were formed.

Photodieldrin was the major metabolic product in the microbes from dieldrin-contaminated soils and lake-bottom silt (Table 1).

To identify the major metabolic product, photodieldrin was prepared by the method of Rosen *et al.* (5). In addition, an authentic sample was obtained from Rosen. The major microbial metabolic product was first isolated by thin-layer chromatography and then compared to the authentic photodieldrin with four different solvent systems: ether-hexane (1 : 1) (Fig. 1); acetone-hexane (1 : 4); methylene chloride-carbon tetrachloride (1 : 1); and benzene-ethyl acetate (3 : 1). The R_F values of photodieldrin under the above conditions were 0.47, 0.46, 0.46, and 0.82, respectively.

Also two different gas-liquid chromatography systems were employed to identify the major metabolic product. The columns used were a 3 percent SE30 and a 6 percent QF 1 (on Chromosorb W, 1.5 m by 0.3 cm) at 180°C with a nitrogen flow rate of



30 ml/mm. A Varian-Aerograph gas chromatographic system (model 1848) and an electron-capture detector were used for this purpose. All the chromatographic matching tests indicated that the major microbial metabolic product was identical to photodieldrin.

The presence of photodieldrin in environments has been shown (4, 5) although the formation of the same end product by environmental microorganisms has not been suspected. More information on the toxicological properties of photodieldrin is needed, since the chances are great that this compound can form from dieldrin in various water and soil systems.

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References and Notes

1. IUPAC Commission on Terminal Residues and H. Eagan, *J. Assoc. Offic. Anal. Chem.* **52**, 299 (1969).
2. C. A. Edwards, *Res. Rev.* **13**, 83 (1966).
3. R. B. Finley and R. E. Pillmore, *Amer. Inst. Biol. Sci.* **13**, 41 (1963); R. P. Miskus, D. P. Blair, J. E. Casida, *J. Agr. Food Chem.* **13**, 481 (1965).
4. J. Robinson, A. Richardson, B. Bush, K. E. Elgar, *Bull. Environ. Contam. Toxicol.* **1**, 127 (1966).
5. J. D. Rosen, D. J. Sutherland, G. R. Lip-ton, *ibid.*, p. 133.
6. J. D. Rosen and D. J. Sutherland, *ibid.* **2**, 1 (1967).
7. M. A. Q. Khan, J. D. Rosen, D. J. Sutherland, *Science* **164**, 318 (1969).
8. F. Matsumura and G. M. Boush, *ibid.* **156**, 959 (1967).
9. F. Matsumura, G. M. Boush, A. Tai, *Nature* **219**, 965 (1968).
10. E. B. Fred and S. C. Waksman, *Laboratory Manual of General Biology* (McGraw-Hill, New York, 1928).
11. Supported in part by a PHS grant FD-00250, and research grant No. 2-04-4200 from the Department of Natural Resources, State of Wisconsin. We thank J. O. Nelson for gas-liquid chromatography analyses.

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Immunoglobulin Structure: Amino Terminal Sequences of Kappa Chains from Genetically Similar Mice (BALB/c)

Abstract. *The amino terminal portion of 20 kappa chains from the highly inbred BALB/c mouse has been examined on an automatic protein sequencer. These proteins can be divided into at least nine groups (subgroups) based on sequence patterns which are so distinct that each subgroup is probably encoded by at least one germ-line gene. The subgroups of mouse kappa chains are generally quite different from those of human kappa chains.*

Remarkable advances have been made toward an understanding of the genetic basis of antibody diversity and evolution through the amino acid sequence analysis of homogeneous immunoglobulins produced by plasmacytomas in man (1, 2). These studies have shown that the immunoglobulin molecule is composed of light (~ 23,000 daltons) and heavy (~ 55,000 daltons) chains which are disulfide-linked. Man produces two types of light chains (λ and κ). The human κ chain has been most thoroughly studied and serves here as a model for all immunoglobulin chains (2). The amino terminal half of the κ chain (~ residues 1 to 107) is designated the variable (V) region (3) because it is different for each myeloma protein studied (> 50). The carboxy terminal half of this chain (~ residues 108 to 214) is designated the common (C) region and is invariant in all human κ chains apart from a single genetic polymorphism (4). The V region sequence diversity in human light chains is impressive. More than 50 human κ and 50 human λ chains have been examined, and each pro-

tein appears to be different from all the others (5). This diversity, however, must be viewed with caution as the human and most other species whose immunoglobulins have been studied are outbred populations and as such have substantial genetic polymorphism (6).

For this reason, we have examined the κ chains from genetically similar individuals of the BALB/c strain of mice that have been consecutively brother-sister mated through more than 110 generations (7). Some information has been published on BALB/c κ chains. The nearly complete amino acid sequences from two κ chains, M-41 and M-70, are available (8) as well as fragmentary sequences from a number of others (9).

We report here our efforts to further characterize the nature and extent of V region variability in this inbred strain by examining the amino terminal sequences of 20 myeloma κ chains and the κ light chains prepared from a pool of mineral oil-induced ascites (Fig. 1) (10). The G, H, and F (IgG, IgH, and IgF) proteins were chosen because of

Subgroup Protein Class		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
V _K I	41	BJ	Asp Ile	Gln Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ser Pro	Ser Ser	Leu Ser	Ala Ser	Leu Ser	Leu Gly	Gly Glu	Arg Val	Ser Thr	Leu Thr	Cys					
	173	IgG	Asp Ile	Glx Met	Thr Thr	Gln Thr	Gln Thr	Ser Thr	Ser Thr	Ser Ser	Leu Ser	Ala Ser	Leu Ser	Leu Gly	Asp ()	Val Thr	Ile							
	31c	IgF	Asp Ile	Gln Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Ser	Ala Ser	Ala Ser	Val Ser	Val Gly	Glu Arg	Val Thr	Ile Thr	Cys						
	23	IgF	Asp Ile	Gln Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Ser	Ala Ser	Val Ser	Val Ser	Val Gly	Glx ()	Val Thr	Ile							
	149	BJ	Asp Ile	Gln Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Asp Tyr	Leu Ser	Ala Ser	Ala Ser	Val Ser	Val Gly	Glu Thr	Val Thr	Ile Thr	Cys						
V _K II	70	IgF	Asp Ile	Val Leu	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Ser	Ala Val	Val Ser	Leu Ser	Leu Gly	Gln Arg	Ala Thr	Ile Ser	Cys						
	321	BJ	Asp Ile	Val Leu	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Ser	Ala Val	Val Ser	Leu Ser	Leu Gly	Gln Arg	Ala Thr	Ile Ser	Cys						
	63	BJ	Asp Ile	Val Leu	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Ser	Ala Val	Val Ser	Leu Ser	Leu Gly	Gln Arg	Ala Thr	Ile Ser	Cys						
V _K III	603*	IgA	Asp Ile	Val Met	Thr Thr	Glx Thr	Glx Thr	Ser Pro	Ser Pro	Ser Ser	Leu Ser	Val Ser	Ala Ser	Ala Gly	Glx ()	Val Thr	Met Ser	Cys						
	870*	IgA	Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ser Pro	Ser Ser	Leu Ser	Val Ser	Ala Ser	Ala Gly	Glu Lys	Val Thr	Met Ser	Cys						
	384	IgA	Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ser Pro	Ser Ser	Leu Ser	Val Ser	Ala Ser	Ala Gly	Glu Lys	Val Thr	Met Ser	Cys						
	467*	IgA	Asp Val	Leu Met	Thr Thr	Gln Thr	Gln Thr	Thr Pro	Leu Ser	Leu Ser	Leu Pro	Val(Ser)Leu	Val(Ser)Leu	Gly Asp	Glu Ala	() Ile(Ser)Cys								
V _K IV	47	IgF	Glu Val	Val Met	Thr Thr	Gln Thr	Gln Thr	Thr Pro	Leu Ser	Leu Ser	Leu Ala	Val(Ser)Leu	Val(Ser)Leu	Gly ()	Glx Ala(Ser)									
	37	IgH	Asp Val	Leu Met	Thr Thr	Gln Thr	Gln Thr	Thr Pro	Leu ()	Leu Pro	Val(Ser)Leu	Val(Ser)Leu	Gly Asp	Glu Ala(Ser)										
	843	BJ	Asp Val	Val Met	Thr Thr	Gln Thr	Gln Thr	Thr Pro	Leu Thr	Leu Thr	Leu Ser	Val Thr	Ile Thr	Ile Gly	Glu Pro	Ala () Ile Ser	Cys							
V _K V	674	BJ	Asp Val	Val Met	Thr Thr	Gln Thr	Gln Thr	Thr Pro	Leu Thr	Leu Thr	Leu Ser	Val Thr	Ile Thr	Ile Gly	Glu Pro	Ala () Ile Ser	Cys							
	8*	IgA	Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Thr Phe	Leu Ala	Val Thr	Ala Val	Thr Thr	Ala Ser	Lys Lys	Val Thr	Ile Ser	Cys						
V _K VI	15	IgA	Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Thr Phe	Leu Ala	Val Thr	Ala Val	Thr Thr	Ala Ser	Lys Lys	Val Thr	Ile Ser	Cys						
	773	BJ	Glu Thr	Thr Val	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Thr Phe	Leu Ala	Val Thr	Ala Val	Thr Thr	Ala Ser	Lys Lys	Val Thr	Ile Ser	Cys						
V _K VII	265	BJ	Glu Thr	Thr Val	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Thr Phe	Leu Ala	Val Thr	Ala Val	Thr Thr	Ala Ser	Lys Lys	Val Thr	Ile Ser	Cys						
	LPC-1	IgG	Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ser Ser	Met Gln	Ala Ser	Ile Ser	Ile Gly	Glu Lys	Val Thr	Ile Ser	Cys							
V _K VIII	167*	IgA	Asp Ile	Val Ile	Thr Thr	Gln Thr	Gln Thr	Asx Glu	Leu Ser	Asp Pro	Val Thr	Ser Ser	Gly Glu	() Val										
	Pool		Asp Ile	Val Met	Thr Thr	Gln Thr	Gln Thr	Ser Pro	Ala Ser	Leu Thr	Ser Ser	Leu Thr	Ser Ser	Leu Thr	Ser Ser	Leu Thr	Ser Ser	Leu Thr	Ser Ser					
V _K IX			Glu	Gln Leu																				
Pool																								

Fig. 1. The amino terminal sequences of 22 BALB/c κ light chains. These chains are divided into subgroups I to IX. The members of a given subgroup resemble one another much more than the proteins of other groups. The pooled light chains were obtained from a mineral oil-induced ascites fluid. The class indicates the derivation of the light chain, whether from an IgA, IgB, IgH, IgF serum protein or directly from the urine (Bence Jones proteins). The data from proteins M-41 and M-70 are taken from (8). Parentheses indicate that the residue was lost or recovered in low yield (18). *Proteins with antibody-like activity to antigens containing phosphorylcholine (32). †Proteins with antibody-like activity to various *Salmonella* O-group antigens (11).

their availability, whereas the A (IgA) proteins were selected because each possessed antibody-like activity (11, 12). About 10 mg of each protein was examined in the Beckman 890 protein sequencer by a program similar to that described by Edman and Begg (13). The resulting phenylthiohydantoin (PTH) amino acids were examined on a Varian model 1840 gas chromatograph (14). The PTH amino acids were hydrolyzed to free amino acids (6N HCl, 150°C, 20 hours) (15) and analyzed on a Beckman 121 amino acid analyzer or by high-voltage paper electrophoresis (16). Trypsin and in some cases thermolysin peptide maps were prepared from each protein (17, 18). All of the C region peptides were identified in one κ chain (M-321). This greatly facilitated subsequent comparisons of V region peptides.

The amino terminal sequences for 22 BALB/c κ chains are given in Fig. 1. This includes two sequences that have been published (M-70 and M-41) (8). Of 22 κ chains, 16 differ in sequence. Proteins with related sequences are placed together in seven subgroups. The unpaired proteins LPC-1 and M-167 probably constitute two additional subgroups. To facilitate a comparison of the variation among subgroups, prototype sequences were determined for each subgroup by choosing the residue at each position which is present in most of the chains (2, 19). The amino terminal sequences for nine mouse and three human V_{κ} prototype sequences were translated into nucleotide sequences which were then compared. The mouse prototype sequences are about as distinct from one another (range 7 to 17 nucleotide differences; average 12 differences) as their human counterparts are from one another (range 11 to 14; average 13). Human κ chains from differing subgroups differ from one another by 40 to 50 percent of their V region sequence (2); thus the percentage difference in the amino terminus of two prototype sequences (23 residues) roughly reflects that seen throughout the entire chain. The magnitude of nucleotide variation is so great among individual human prototype sequences that it has been concluded that at least one germ line gene is required for each (1, 2, 20). The same appears to be true of the BALB/c κ prototype sequences. Since these mice are inbred, each mouse has the potential to generate all of the V_{κ} diversity seen in the BALB/c population as a whole. Thus even with this limited

sample of κ chains, it appears that at least nine germ-line genes encode BALB/c V_{κ} diversity.

In our analysis, the mouse κ chains have more prototype sequences than their human counterparts (nine as opposed to three) (2, 21). Two possible explanations may be offered. First, the plasma cell tumors in the mouse are derived from a different set of precursor cells. For example, the ratio of IgA to IgG of tumor proteins is clearly different in the two species—being roughly 65/35 in the mouse and the inverse in man (22). Accordingly, different plasma cell pools may be available for malignant transformation in the two species. Second, perhaps the myeloma process accurately reflects a greater diversity of κ chains in mouse as compared with man. The ratio of λ to κ in the mouse is 3/97 (23) as compared to 1/2 in man (24). Furthermore, the variation in sequence of the V region in ten mouse λ chains is very limited (one to three substitutions) (25). Perhaps the mouse has had to expand its V_{κ} gene population to compensate for a lack of V_{λ} genes.

The mouse subgroups, apart from $V_{\kappa 1}$, are as different from their human counterparts as they are from each other. As others have noted (26), the amino acid alternatives at each of the first 23 residues are remarkably similar in both species. The linked subgroup sequences, however, are quite different. Thus, the subgroup prototype sequences are species specific. If a separate gene encodes each distinct V region in the BALB/c mouse, an apparent problem arises in explaining how multiple genes can evolve to generate species-specific subgroups. The existence of species-specific subgroups probably reflects a mechanism for rapid gene expansion-contraction which operates on different germ-line genes in different evolutionary lines. Homologous but unequal crossing-over may expand different sets of genes in different evolutionary lines, and accordingly species-specific subgroups would arise. There are precedents for such a mechanism of multigene evolution [see (27) for a more thorough discussion].

Although it is generally agreed that a distinct germ-line gene is required for each V region subgroup or prototype sequence (1, 2, 28), a controversy exists as to whether intrasubgroup variation is encoded in the germ line (germ-line theory) or whether it is generated somatically by mutation or recombination (somatic theories). Unfortunately,

at this time insufficient data are available to make detailed comparisons of the intrasubgroup variation of BALB/c κ chains. Variation in the amino terminal 23 residues is found in subgroups I, IV, and V. Indeed the decision to place a protein within a subgroup was at times somewhat arbitrary (for example, M-173 differs from the prototype sequence of the $V_{\kappa 1}$ proteins by three residues), and, with additional sequence information, certain of these proteins may constitute separate subgroups. Proteins in subgroups II and III with identical amino terminal sequences (Fig. 1) had unequivocal V region peptide differences on tryptic peptide pattern analyses. Proteins in subgroups VI and VII appeared to be very similar, if not identical, to one another (29–31).

Because of possible selection by the myeloma process, the sample shown here does not necessarily express all of the region diversity that the BALB/c mouse genome is capable of generating. In Fig. 1 are given the major κ chain residue alternatives for positions 1 to 11 in the amino terminus of pooled light chains from BALB/c mice. These chains which were obtained from a mineral oil-induced ascites clearly have sequence diversity not present in the myeloma κ chain pool. Although the residue alternatives correspond qualitatively to the major residue alternatives in the myeloma pool (apart from the Phe and Leu at positions 9 and 10, respectively), there are some striking quantitative differences. For example, at position 1 the ratio of Asp to Glu in light chains from the ascites pool is 2/1 and from the myeloma pool it is 6.7/1. Furthermore, the Glu at position 1 in the myeloma pool is associated with Val (M-47) or Thr (M-773 and M-265) at position 2. Neither of these alternatives is seen at a significant level at position 2 in the ascites pool. Hence, the Glu at position 1 in the light chains from the ascites pool is probably representative of a normal V region subgroup, which is not selected by the myeloma process. Therefore any conclusions based on the diversity found in myeloma proteins must be tempered by the realization that the myeloma pool represents only a subset (of unknown size) of true κ chain diversity in the BALB/c mouse.

These results clearly suggest that a multigene system encodes the V regions of the mouse κ chain. Although these studies are still incomplete, the BALB/c system does offer considerable promise for deciphering the mechanism

of antibody diversity and the mechanism of evolution in a multigene system. Indeed, it seems likely that other complex mammalian systems should employ a similar strategy for information storage and transfer, and hence the immune system should serve as a useful general model for differentiation in higher organisms

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References and Notes

1. F. W. Putnam, *Science* **163**, 633 (1969); G. M. Edelman and W. E. Gall, *Annu. Rev. Biochem.* **38**, 415 (1969); J. Sterzl and H. Rha, Eds., *Symposium on Developmental Aspects of Antibody Formation and Structure* (Academic Press, New York, in press); C. Milstein and J. Pink, *Progr. Biophys. Mol. Biol.* **21**, 211 (1970).
2. L. Hood and D. W. Talmage, *Science* **168**, 325 (1970).
3. The subgroup designations for immunoglobulin V (variable) regions are those proposed by the Conference on Nomenclature for Animal Immunoglobulins, Prague, June 1969. See *World Health Organ. Bull.* **41**, 975 (1969). The abbreviations for amino acid residues are Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Asx, aspartic acid or asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Glx, glutamic acid or glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; and Val, valine.
4. C. Baglioni, L. A. Zonta, D. Cioli, A. Carbonara, *Science* **152**, 1517 (1967); C. Milstein, *Biochem. J.* **101**, 338 (1966); C. Ropartz and J. Lenoir, *Nature* **189**, 586 (1961); A. G. Steinberg, J. A. Wilson, S. Lanset, *Vox Sang.* **7**, 151 (1962); W. D. Terry, L. Hood, A. G. Steinberg, *Proc. Nat. Acad. Sci. U.S.* **63**, 71 (1969); W. J. Dreyer and J. C. Bennet, *Proc. Nat. Acad. Sci. U.S.* **54**, 864 (1965); W. J. Dreyer, W. R. Gray, L. Hood, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 353 (1967).
5. R. Quattrocchi, D. Cioli, C. Baglioni, *J. Exp. Med.* **130**, 401 (1969); G. P. Smith, thesis, Harvard University (1969).
6. J. L. Hubby and R. C. Lewontin, *Genetics* **54**, 577 (1966); C. Willis, *Proc. Nat. Acad. Sci. U.S.* **61**, 937 (1968).
7. The animals were raised at the NIH Animal Breeding Section, where the genealogical record is kept.
8. W. R. Gray, W. J. Dreyer, L. Hood, *Science* **155**, 465 (1967).
9. L. Hood, W. R. Gray, W. J. Dreyer, *Proc. Nat. Acad. Sci. U.S.* **55**, 826 (1966); R. Perham, E. Appella, M. Potter, *Science* **154**, 391 (1966); F. Melchers, *Biochemistry* **8**, 938 (1969).
10. Urinary light chains (Bence Jones proteins) were isolated by ammonium sulfate precipitation from urine, followed in some cases by ion exchange chromatography (carboxymethyl Sephadex). Serum light chains were isolated from completely S-carboxymethylated IgA, IgG, IgH, and IgF myeloma proteins by gel filtration (Sephadex G-100 in 8M urea and 0.1M acetic acid).
11. M. Potter, *Fed. Proc.* **29**, 85 (1970).
12. —, R. Lieberman, L. Hood, D. J. McKean, *ibid.* **29**, 437 (1970).
13. P. Edman and G. Begg, *Eur. J. Biochem.* **1**, 80 (1967).
14. J. J. Pisano and T. J. Bronzert, *J. Biol. Chem.* **244**, 5597 (1969).
15. H. O. Van Orden and F. H. Carpenter, *Biochem. Biophys. Res. Commun.* **14**, 399 (1964).
16. W. J. Dreyer and E. Bynum, *Methods Enzymol.* **11**, 32 (1967).
17. A. M. Katz, W. J. Dreyer, C. B. Anfinsen, *J. Biol. Chem.* **234**, 2897 (1959).
18. A detailed analysis of these peptide map comparisons has been made, and yield data for the sequenator analyses have been obtained (D. J. McKean, L. Hood, M. Potter, in preparation).
19. C. Milstein, *FEBS Lett.* **2**, 301 (1969).
20. L. Hood, W. R. Gray, B. G. Sanders, W. J. Dreyer, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 133 (1967); H. Niall and P. Edman, *Nature* **216**, 262 (1967); C. Milstein, *ibid.*, p. 330; O. Smithies, *Science* **157**, 267 (1967).
21. There is probably a fourth V_{H1} subgroup in man which cannot be readily distinguished from the V_{H1} subgroup in the 23 residues of the amino terminal [see (2)].
22. M. Potter, *Midwinter Symposium on Biochemistry*, Miami, J. Schulz, Ed. (North-Holland, New York, in press); R. Bachmann, *Scand. J. Clin. Lab. Invest.* **18**, 273 (1966); J. R. Hobbs, *Brit. Med. J.* **3**, 699 (1967); J. Hallen, *Acta Med. Scand. Suppl.* No. 462 (1966), pp. 1-127; E. F. Osseman, R. A. Rifkind, K. Takatsuki, D. P. Lawlor, *Ann. N.Y. Acad. Sci.* **113**, 627 (1964).
23. K. R. McIntire and A. M. Pouse, *Fed. Proc.* **29**, 704 (1970).
24. J. L. Fahey, *J. Immunol.* **91**, 438 (1963).
25. M. Weigert, I. Cesari, S. Yonkovich, M. Cohn, *Nature*, in press. Similar observations were made by E. Appella and R. N. Perham, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 37 (1967).
26. T. T. Wu and E. A. Kabat, *J. Exp. Med.*, in press.
27. L. Hood, K. Eichmann, H. Lackland, R. M. Krause, J. Ohms, *Nature*, in press.
28. J. Gally and G. M. Edelman, *Nature*, **227** 341 (1970); N. K. Jerne, *Proc. Nat. Acad. Sci. U.S.*, in press; G. P. Smith, thesis, Harvard University (1969); N. Hilschmann, H. U. Barnikol, M. Hess, B. Langer, H. Ponstingl, M. Steinmetz-Kayne, L. Suter, S. Watanabe, in *Current Problems in Immunology* (Springer-Verlag, New York, 1969), p. 69; M. Cohn, in *Rutgers Symposium on Nucleic Acids in Immunology*, O. J. Plescia and W. Braun, Eds. (Springer-Verlag, New York, 1968), p. 671.
29. One of the most interesting observations made in this study is the possible sequence identity of two pairs of light chains. Bence Jones proteins M-793 and M-265 are identical by individual antigenic specificity (30), by thermolysin and tryptic peptide patterns and in their amino terminal 23 residues. If these proteins are identical, a direct estimate of the size of the mouse myeloma V_{H1} pool size may be forthcoming (31). The failure to find pairs of proteins with similar, if not identical, sequences in the human population (5) may be due to genetic polymorphism which would render impossible any estimate of the size of the V_{H1} pool.
30. P. Periman, unpublished results. The protein M-773 was the gift of Dr. R. McIntire, and MPC-37 and MPC-47 were the gift of Dr. R. Asofsky.
31. Knowing the number of κ chains examined in order to obtain two identical sequences, one can make a statistical calculation to determine the number of different sequences present in the κ pool [See S. Cohen and C. Milstein, *Advan. Immunol.* **7**, 1 (1967)].
32. Two groups of immunoglobulins with antibody activity have been studied. Proteins 8, 15, 167, and 603 have antibody activity to antigens that contain phosphorylcholine (11, 12). Proteins 870, 384, and 467 have activity against *Salmonella* O antigens (11). The light chains from these proteins fall into three subgroups. Indeed, light chains from immunoglobulins that identify different antigenic species are present in the V_{H1} subgroup. Thus, no simple correlation between antibody activity and V region subgroups is apparent [See A. Kaplan and H. Metzger, *Biochemistry* **8**, 3944 (1969); S. Cohen and A. Cooper, *Immunology* **15**, 93 (1968)].

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Bare Zone between California Shrub and Grassland Communities: The Role of Animals

Abstract. Between shrub and grass communities in coastal California there is a zone that is normally bare of vegetation. Previous studies have emphasized the role of volatile inhibitors of plant growth in producing this bare zone. However, there is a concentration of feeding activity by rodents, rabbits, and birds in this zone; if this activity is prevented by means of wire-mesh exclosures, annuals grow in the bare zone. Thus, animal activity is sufficient to produce the bare zone.

There is a characteristic bare zone adjacent to many shrubs that are members of the California chaparral and coastal sage communities (1). This zone is particularly obvious where a stand of these shrubs is immediately adjacent to a grassland area. Previous studies have emphasized the role of volatile inhibitors of plant growth produced by the shrubs, particularly terpenes, as being the main factor in the production and maintenance of this zone (2). That cattle grazing and trails could be responsible for this zone (3) has been discounted by Muller and others (4). Although Muller and co-workers acknowledge that small mammals and birds may be involved in the maintenance of the bare zone, they consider that plant toxins are mandatory for production of a bare zone (4, 5).

I have examined whether animal activity alone could account for the bare zone. This possibility has been suggested by J. R. Rood for a thorn bush association in Argentina. He states that the bare zone adjacent to the plants *Schinus fasciculatus* and *Cordia microphylla* is caused by the hystricomorph rodent *Microcavia australis* (6).

In California the chaparral and coastal sage shrubs form excellent cover for rodents, rabbits, and birds. The adjacent grassland provides poor cover for most of these animals yet furnishes an excellent food supply for grazers and seed eaters. One would expect then that feeding activity of these small animals would be concentrated in grassland areas immediately adjacent to stands of shrubs. To see if increased