vironment from a wide variety of biological sources as well as from petroleum released by natural seeps and man's activities, and that branched and cyclic alkanes accumulate at the interface as a result of a complex combination of physical and biological influences. The data that have been collected so far show that the types and amounts of hydrocarbons in the surface film along the northern Gulf Coast have remained fairly constant during the study period.

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Specificity of Antibodies: Primary Structural Basis of Hapten Binding

Abstract. *The primary structure of the 83 residues of the NH_2-terminus of the V_H region was determined for each of three different antibodies to hapten which were produced in inbred guinea pigs. Each antibody had a different and distinctive primary structure within each of the two "hypervariable" regions (Hv1 and Hv2) included in the analyzed part of the variable region of the heavy chain. The sequences of Hv1 and Hv2 in the three antibodies were either unique or of restricted variability compared with those of "normal" immunoglobulin G2. Further implication of Hv1 and Hv2 in contributing to ligand-binding specificity of antibodies came from the placement of residues modified by affinity labeling reagents in these hypervariable regions.*

The accurate correlation of the primary structures of antibodies with their ligand-binding specificities has been a long-term goal of immunologists. This correlation would allow them (i) to better describe the "active" sites of antibodies and eventually place amino acid residues contributing to antigen binding in their actual three-dimensional array; (ii) to infer the magnitude of genetic information for amino acid sequences of antibodies transmitted through the germ line; and (iii) to use distinctive sequences of antibodies as markers for lines of B (bone marrow derived) lymphocytes and plasma cells in order to better understand the regulation of the immune response in vivo.

We used inbred guinea pigs as donors of antibodies specific for the dinitrophenyl (DNP), *p*-azobenzenearsonate (ARS), or *p*-azobenzenetrimethylammonium (TMA) haptens to circumvent that genetic polymorphism present in an outbred population which is expressed as allotypic differences among proteins. Different individuals, immunized with the same antigen according to the same regimen, might be expected to produce antibodies with the same or similar binding-site structure because inbred animals inherit the same structural genes for antibodies and any alterations of these genes during somatic cell division followed by clonal selection would occur within animals with the same genetic background.

In a primary structural analysis of the heavy chain (γ_2 chain) from the most abundant isotype of guinea pig antibody, immunoglobulin G2 (IgG2), we isolated and rigorously aligned eight fragments from a CNBr digest of "normal" γ_2 chain, accounting for its entirety (*1*). Since "normal" IgG2 is a mixture of molecules with different binding specificities, we reasoned that fragments with a single amino acid sequence obtained from such a pool would not be directly involved in determining antigen binding. On the other hand, variability at a given residue position might be a marker for those segments of the molecule imparting antigen-binding specificity. A single primary structure was found for five COOH-terminal fragments, totaling 306 residues (*2*), and was expected because others have characterized two "active" fragments of antibody, Fab and Fv, each of which retained a ligand-binding site but lacked COOH-terminal regions of the parent molecules (*3*).

Our attention was focused on three CNBr fragments of guinea pig γ_2 chain comprising V_H, the NH_2-terminal quarter of heavy chain wherein sequences of myeloma proteins differ, because they contained those residue positions specifically modified by affinity labeling reagents (*4*). These chemically reactive analogs of antigen determinants are concentrated in binding sites of antibodies where they may react covalently with an appropriate amino acid residue, provided one is situated close enough to the rigidly bound reagent (*5*). When we used the affinity labeling reagents MNBDF (*6*), BADL, and BAAT to modify anti-DNP or anti-ARS, the specifically substituted residues were found within C-1-n, C-1-a_1, and C-1-a_2 (*7*). These three fragments contain the ~ 140 residues of the NH_2-terminus of γ_2 chain (*1*), and when derived from "normal" heavy chain each contains all or most of one short segment of highly variable primary structure—residues N31 to N35, N48 to N59, and N99 to N118—called "hypervariable" regions Hv1, Hv2, and Hv3, respectively (*8*). These Hv regions are surrounded by sections of V_H which appear to each have a single sequence, except where alternative residues have been detected at N2, N16, and N79 (Fig. 1). Analogous "hypervariable" segments in human and murine light (L) chains have been detected by comparison of numerous sequences of V_L region from

myeloma proteins (*9*). Comparison of the first few sequences determined for V_H of human myeloma proteins indicated some segments of "hypervariability" (*10*) which were more clearly defined when more primary structural data for many myeloma proteins became available and after the V_H sequences were arbitrarily segregated into subgroups on the basis of sequence relatedness (see *11*). However, myeloma V_H sequences show variation outside the Hv regions and extensive differences between two V_H regions from proteins of different subgroups. The primary structure of guinea pig V_H outside of the Hv regions is more restricted in heterogeneity as compared to the homologous part of human myeloma heavy chains. Particularly, the many subgroups of human V_H, defined by linked sequence variations outside Hv regions, are apparently not expressed in the guinea pig. Thus we expected that correlations of sequence with antigen-binding specificity would be more easily obtained with antibodies from inbred guinea pigs.

If Hv regions determine ligand-binding specificity, these should have a single or restricted primary structure in particular antibodies compared with "normal" IgG2. Furthermore, each specificity would be expected to be associated with its own distinctive Hv primary structures. The primary structures of N1 to N83 of V_H, including Hv1 and Hv2, have been determined for purified antibodies of three different specificities (*12*). The results of these analyses (Fig. 1) indicated (i) that the three antibodies to haptens have a much more restricted primary structure in Hv1 and Hv2 than "normal" IgG2 does; (ii) that the primary structures of both Hv1 and Hv2 are different for each antibody to hapten and correlate with ligand-binding specificity in that each preparation raised to the same determinant contained the same distinctive residues at "variable" positions; and (iii) those sections of V_H surrounding Hv regions from N1 to N83 have the same single amino acid sequence in all three antibodies to haptens and in "normal" IgG2, except at positions N2, N16, and N79. These observations suggest that the Hv regions have a major role in determining the antigen-binding specificity of antibodies. This suggestion is further supported by the placement of residues modified by affinity labeling within or immediately adjacent to Hv1 and Hv2 at N33, N59, and N60 (Table 1 and Fig. 1). The tyrosyl residue at N33, modified by BADL, and the lysyl residue at N59, substituted by BAAT, are distinctive of the anti-DNP and anti-ARS specificities, respectively. Finally, analysis of the three-dimensional structure of an Fab fragment from a human myeloma protein by x-ray crystallography (*13*) reveals a shallow trough made up of residues in positions corresponding to those in the three Hv segments apparent in guinea pig V_H and in the analogous hypervariable segments defined in human and mouse L chains (*9*). Thus the Hv regions appear to be close to each other within the antibody molecule in its native configuration.

Table 1. Residue positions of antibodies modified by affinity labeling reagents within or adjacent to hypervariable regions.

Region	Anti-DNP		Anti-ARS
	MNBDF	BADL	BAAT
Hv1	Tyr 32/33	Tyr 33	Trace
Hv2	Tyr 60	Trace	Lys 59
Hv3	Tyr ?	Trace	Trace

Our data suggest that the structural genes for many V_H regions with different antigen-binding specificities are inherited and that different individuals and different precursor cells in the same animal can express the same or very similar V_H structural genes. As Fig. 1 indicates, antibodies to haptens produced in inbred guinea pigs are not homogeneous, but many of the antibodies of a given specificity have the same amino acid at a given residue position in Hv1 and Hv2. Others have found, using immunochemical methods, that different individuals of certain inbred strains of mice make significant fractions of anti-ARS or antibody to phosphorylcholine, which have the same or closely similar binding-site structures (*14*). Further support for inheritance of V_H genes for particular antibody specificities comes from the repeated occurrence, in a selectively bred rabbit family, of antibodies to carbo-

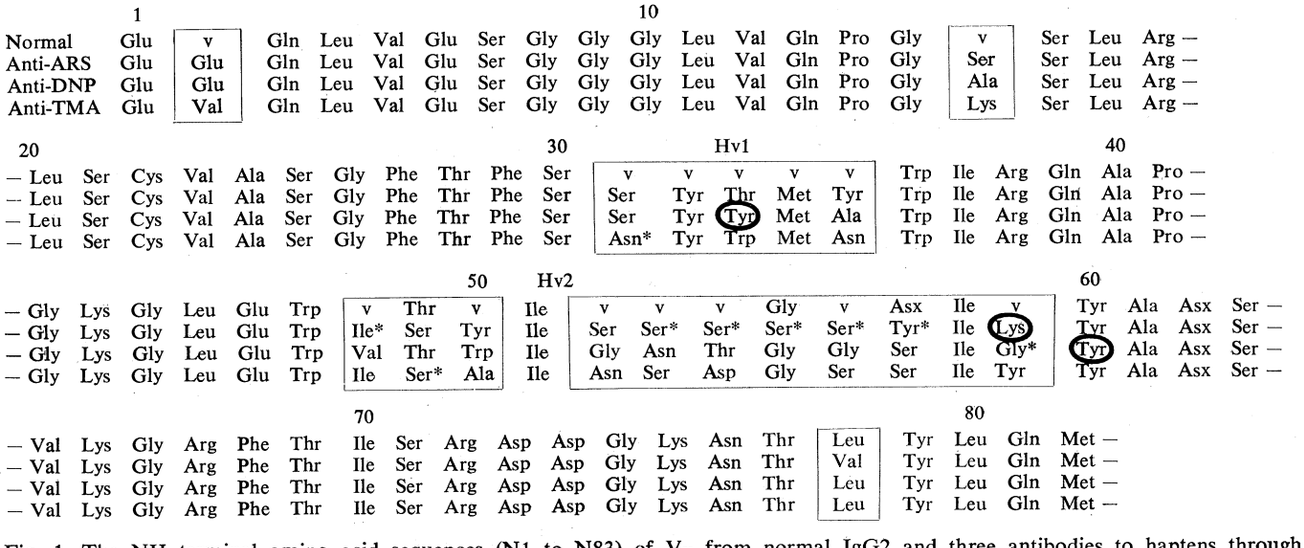

Fig. 1. The NH_2-terminal amino acid sequences (N1 to N83) of V_H from normal IgG2 and three antibodies to haptens through hypervariable regions Hv1 and Hv2. Residue positions having alternative amino acids are shown in boxes. Those residues modified by affinity labeling reagents are circled (Table 1). A residue position bearing an asterisk shows the predominant amino acid although other amino acids have been detected. The v positions shown in the sequence of the normal γ_2 chain indicate variable positions at which two or more alternatives have been identified.

hydrate with the same or closely related site structures (*15*). To define a minimum number of structural genes for V_L, Cohn and his colleagues considered that only amino acid interchanges outside "hypervariable" regions may be ascribed to different germ line genes, while changes within these regions may arise due to alteration of V genes during somatic cell division (*16*). Each of the three guinea pig antibodies to haptens has a distinctive pattern of residues at those variable positions outside the Hv regions, at N2, N16, and N79. Thus a minimum of three V_H genes seems necessary for the three ligand-binding specificities. However, since each of these V_H genes apparently results in a predominant product having a distinctive sequence in Hv1 and Hv2 as well, such coarse reproducibility in expression by different individuals would seem most simply accomplished by encoding the distinctive Hv1 and Hv2 regions in the same V_H genes. The analysis of Hv3 from these antibodies, which seems to be less restricted in primary structure than Hv1 and Hv2, may uncover variability including useful markers for assessing whether alterations in V_H genes may occur during the proliferation of plasma cell precursors. Meanwhile, the distinctive sequences of Hv1 and Hv2 are themselves proving useful markers for following the fate of clones of cells expressing the same or similar V_H genes in animals caused to become tolerant or unresponsive, deviated with respect to isotype of antibody produced, or idiotype suppressed in order to probe the normal regulation of the immune response.

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N′-Nitrosonornicotine in Tobacco

Abstract. *N′-Nitrosonornicotine, a potential carcinogen, has been positively identified in unburned tobacco. The amount in commercial U.S. tobacco products is between 1.9 to 88.6 parts per million, one of the highest values of an environmental nitrosamine yet reported. The amount in food and drink rarely exceeds 0.1 part per million. This compound is the first example of a potential organic carcinogen isolated from tobacco.*

A number of *N*-nitroso compounds are carcinogenic or mutagenic (or both) in a wide variety of experimental animals (*1*). This biological activity has been demonstrated repeatedly and, although there is no direct evidence that these compounds are human carcinogens, they should be regarded as potential hazards. Because *N*-nitrosamines are easily formed by the reaction of secondary amines with nitrite and, to a lesser extent, by the reaction of tertiary amines with nitrite (*2*) and because various environmental sources contain both nitrite and the appropriate amine precursors, an intensive search has begun for *N*-nitrosamines in food, drink, and other materials ingested or inhaled by man (*3*). It is now the consensus of opinion that *N*-nitrosamines should be considered as potential health hazards at concentrations of part per billion (ppb, microgram per kilogram) (*4*).

Tobacco smoke is carcinogenic in the experimental animal, but the overall biological activity can be explained only partially by the components isolated to date (*5*). Following the suggestion of Druckrey and Preussmann that nitrosamines may contribute, at least in part, to the observed carcinogenicity (*6*), several investigators have identified varying quantities of volatile nitrosamines in cigarette smoke (*3*, *7*). We have reported the identification of a nonvolatile nitrosamine, *N′*-nitrosonornicotine (NNN), in the unaged smoke of a popular American blended cigarette without a filter tip (85 mm) at a concentration of 137 ng per cigarette (*7*). This compound has also been reported in the smoke of cigarettes made from tobacco rich in nornicotine (*8*). *N′*-Nitrosonornicotine induces in mice multiple pulmonary adenomas with local invasion of the lung and the bronchi (*9*). Since our goal is to identify the source of potential hazardous substances in tobacco smoke and to devise methods to minimize their precursors, we have analyzed unburned tobacco for NNN.

We now report that various types of tobacco products contain NNN at 2 to 90 μg/g (dry weight of the tobacco) [2 to 90 parts per million (ppm)] (Table 1). This is to our knowledge the highest concentration of a positively identified *N*-nitrosamine yet reported in an environmental source. *N*-Nitrosamines in meat, fish, beverages, and related materials rarely exceed 0.1 ppm (*3*).