serum that destroys the neurons and nerve fibers is nondialyzable and therefore is presumably a protein. We have inferred that its site of action is at the somas of the anterior horn cells because when 5-day in vitro cultures were used the ALS serums usually had no effect. In these cases, there was a good outgrowth of nerve fibers which were directly exposed to the ALS serum in the culture medium. However, the center of the explants had not yet flattened out and the anterior horn cell somas were covered by other cells. It was only when the cultures were monolayers and the somas of anterior horn cells were exposed to the culture medium that they were destroyed by the ALS serum.

Fifty-four control serums from 14 different degenerative neurological diseases were tested, and they had no effect on the cultures (Table 1). Of particular interest was the lack of toxicity to anterior horn cells in the serums of the cases of Werdnig-Hoffman disease, which is an inherited degenerative disease of the anterior horn cells of infants and children. From this it would seem that the toxicity of the ALS serums is not due to some general phenomenon secondary to the degeneration of the anterior horn cells in the ALS patients. Antibodies that demyelinate cultures of cerebellum are present in a high proportion of the serums of patients with multiple sclerosis (*3*). Approximately 60 percent of ALS serums also demyelinate these cultures (*3*, *4*), and this is inexplicable, since ALS is not a demyelinating disease. The serums of our cases of multiple sclerosis had no effect on the anterior horn cells in the cultures, indicating that the toxic factor for these cells in ALS serum is not the same as the demyelinating antibodies that have been reported in multiple sclerosis and ALS serums. To our knowledge this is the first demonstration of toxicity to living neurons by serum from a neuronal degenerative disease of man. The significance of our observation in terms of the pathogenesis of ALS can be determined only after the toxic factor has been identified.

Table 1. Neurological diseases used as controls.

Disease	Cases (No.)
Multiple sclerosis	15
Werdnig-Hoffman	6
Guillain-Barré	5
Presenile dementia	5
Huntington's chorea	4
Parkinson's disease with dementia	4
Myasthenia gravis	3
Spinocerebellar degenerations	4
Charcot-Marie-Tooth disease	3
Parkinson's disease	2
Jacob-Creutzfeld disease	1
Carcinomatous neuropathy	1
Polio	1
Total	54

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A Hidden Antigenic Site Localized to the Constant Region of Light Chains of Immunoglobulins

Abstract. *An antigenic site, which was not immunochemically demonstrable in the intact κ light polypeptide chain, was exposed by enzymatic cleavage of the κ chain into its variant half and constant half. This antigenic site located in the constant region the κ chain was detected immunochemically by several antiserums that had specificity for this site. Treatment of an intact κ chain with a dissociating agent resulted in the exposure of the hidden antigenic site, which was as readily detected in the unfolded polypeptide chain as in the isolated constant half.*

The light chains of immunoglobulins can be cleaved specifically into two halves (*1*), the NH_2-terminal variant half (V_L) and the COOH-terminal constant half (C_L). The V_L and C_L have been characterized structurally and immunochemically (*1*, *2*). The amino acid sequence of the V_L of all light chains appears to be unique for each protein, whereas the sequence of the C_L of all light chains of the same type (κ or λ) is virtually identical (*3*). During our immunochemical studies (*4*), which have confirmed the structurally defined (*3*) subgroups of light chains and have differentiated among proteins of the same subgroup, we found that certain antiserums had specificity for a hidden antigenic site localized to the C_L.

To study the immunochemical relation between C_L and intact Bence-Jones protein, we subjected Bence-Jones proteins to limited proteolysis so that some intact protein remained (*1*). Len, a κ Bence-Jones protein, was cleaved selectively; and three components, the C_L, intact Bence-Jones protein, and V_L, were detected by immunoelectrophoretic analysis (Fig. 1A). Three types of antiserums were used in the identification of the C_L and V_L (*1*). When tested with homologous antiserum against Len, the C_L and V_L were antigenically deficient to the intact protein Len. Absorption of the homologous antiserum with a heterologous κ Bence-Jones protein removed the reactivity with antigenic determinants in the C_L, and the resulting antiserum gave a precipitin reaction of identity between the V_L and the intact protein Len. An antiserum prepared against a heterologous κ Bence-Jones protein recognized only antigenic determinants in the C_L and gave a precipitin reaction of identity between the C_L and the intact protein Len.

With an antiserum against Bence-Jones protein, the more typical and previously demonstrated immunochemical relation between the C_L and the homologous Bence-Jones protein had been that the C_L was antigenically deficient to the intact protein. However, certain homologous antiserums were unusual in their reactivity in that they had major specificity for the C_L. These antiserums recognized an antigenic site that was localized in the C_L and that was not exposed or immunochemically recognized in the native or intact κ chain. Furthermore, examination of the reactivity of our numerous antiserums against κ Bence-Jones proteins with the partially cleaved sample of protein Len revealed that with certain heterologous antiserums the intact Bence-Jones protein was antigenically deficient to the C_L. The specificity of these antiserums for a hidden antigenic site that was exposed by cleavage of the intact Bence-Jones protein could be readily demonstrated. Absorption of either a homologous or heterologous antiserum with

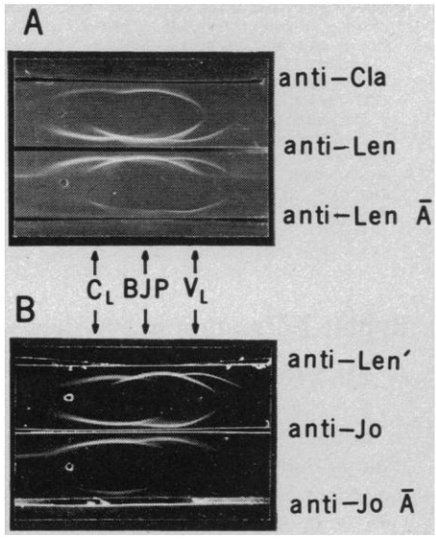

Fig. 1. (A) Detection of the constant half (C_L) and variant half (V_L) produced by pepsin cleavage of a κ Bence-Jones protein (BJP). The antigen wells contained Bence-Jones protein Len subjected to limited proteolysis (*1*). The upper trough contained an antiserum (anti-Cla) prepared against Cla, a heterologous κ Bence-Jones protein; the middle trough contained an antiserum (anti-Len) prepared against the homologous protein Len; and the lower trough contained the homologous antiserum absorbed with a heterologous κ Bence-Jones protein (anti-Len Ā). (B) Immunochemical demonstration of an antigenic site localized to the C_L of the κ light polypeptide chain. The antigen wells contained Bence-Jones protein Len subjected to controlled proteolysis. The upper trough contained a homologous antiserum (anti-Len′), obtained at a later date, which has specificity for a site in the C_L that is not recognized on the intact protein Len. The middle trough contained a heterologous antiserum (anti-Jo), prepared against a κ Bence-Jones protein Jo. This antiserum has specificity for an antigenic site in the C_L and shows the intact protein Len to be antigenically deficient to the C_L of protein Len. The lower trough contained the heterologous antiserum absorbed with intact Len (anti-Jo Ā). The specificity of the antiserum is evident from the precipitin reaction.

intact protein Len removed reactivity with the intact protein Len and left reactivity with the C_L (Fig. 1B).

The hidden antigenic site was exposed by dissolving lyophilized Bence-Jones protein Len in 8*M* urea. The exposure of the site through the unfolding of the molecules was demonstrated immunochemically with an antiserum specific for this determinant (Fig. 2A). In Ouchterlony immunodiffusion analysis in which antiserum specific for the hidden C_L site was used, the urea-treated Len protein gave a precipitin reaction of identity with the C_L Len. Subsequent removal of the dissociating agent by dialysis against 0.15*M* NaCl resulted in the refolding of the protein molecules with concomitant loss in immunochemical reactivity with the specific antiserum. The phenomenon was repeatable; that is, dialysis of the refolded protein molecules against 8*M* urea resulted in the reexposure of the site.

Protein Len belonged to the basic κ chain group κII (*3*, *4*). Representative proteins belonging to the basic κ chain groups κI and κIII were subjected to controlled cleavage and subsequently analyzed immunochemically. The exposure of an antigenic site located in the C_L was readily demonstrable. The antiserums that had specificity for the site were not restricted to antiserums prepared against proteins belonging to a particular κ chain group, but certain antiserums against κI chains, certain antiserums against κII chains, and certain antiserums against κIII chains all had specificity for this hidden site. Furthermore, the hidden antigenic site was readily apparent in κ chains of different Inv allotypes (*3*). The reactivity of antiserum against a κI, Inv (*3*) Bence-Jones protein with selectively cleaved κI, κII, and κIII Bence-Jones proteins is shown in Fig. 2B.

The hidden antigenic site of the C_L was detected immunochemically after a κI Bence-Jones protein had been cleaved with pepsin at 55°C (*1*). Naturally occurring urinary proteolytic enzymes and other endopeptidases, such as trypsin, were used in the cleavage of κ chains; the hidden C_L site was detected immunochemically in each instance.

Specificity for the hidden C_L site was expressed by antiserums prepared against intact Bence-Jones proteins that had been purified, lyophilized, dissolved in 0.15*M* NaCl, and emulsified in Freund's complete adjuvant (*4*), as well as by antiserums against similarly prepared Bence-Jones proteins dissolved in 0.15*M* NaCl and heated to 100°C before emulsification. Immunochemical analyses of the antigens used for immunization revealed that the hidden C_L site was not exposed; only intact Bence-Jones protein was detected. Neither heating the antigen to 100°C nor emulsifying it in Freund's complete adjuvant exposed the hidden site; therefore, the antigens were apparently intact at the time of injection. Since several of our antiserums prepared against intact Bence-Jones proteins have specificity for the hidden C_L site, it appears that this highly antigenic site was exposed in vivo.

Functional antigenic sites that are

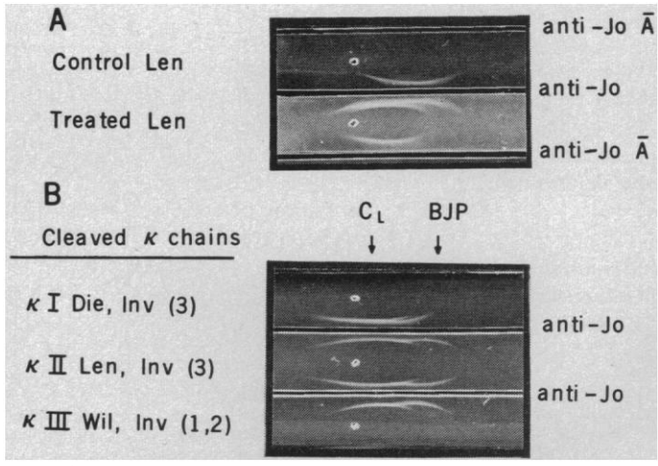

Fig. 2. (A) Immunochemical demonstration of an antigenic site exposed by unfolding the polypeptide chain. The upper antigen well contained Bence-Jones protein Len in 0.15*M* NaCl, and the lower well contained protein Len in 8*M* urea. The upper and lower troughs contained a heterologous antiserum prepared against κI Bence-Jones protein Jo and absorbed with intact protein Jo (anti-Jo Ā). This absorbed antiserum has specificity for the hidden antigenic site localized to the C_L. As evident, the absorbed antiserum did not react with the untreated intact protein Len, but a precipitin arc was formed with the treated protein Len. The middle trough contained the unabsorbed antiserum against the κI protein Jo; this antiserum gave a single precipitin arc with the untreated protein Len. Two precipitin arcs were formed with the treated protein Len. The precipitin arc furthest from the trough represents those molecules in which the hidden C_L site was exposed by unfolding of the polypeptide chain; and the precipitin arc nearest the trough represents undissociated protein Len. (B) Immunochemical detection of an exposed antigenic site common to the C_L of κ chains of different Inv allotypes. The proteins studied were κI Die, Inv(3); κII Len, Inv(3); and κIII Wil, Inv(1, 2). These proteins were subjected to controlled pepsin cleavage and analyzed by immunoelectrophoresis. Each intact Bence-Jones protein (BJP) is clearly antigenically deficient to its respective C_L with the antiserum to κI Jo, Inv(3); this antiserum has specificity for the hidden antigenic site exposed by cleavage.

masked under physiological conditions have been previously demonstrated; for example, certain human serums were found to contain antibodies with specificity for a hidden antigenic determinant on the immunoglobulin G molecule which could be revealed by pepsin digestion (*5*). In rabbit serums, agglutinators were found with specificity for buried antigenic determinants on the Fab fragment of enzymatically digested rabbit immunoglobulin (*6*).

Although the functional significance of the hidden C_L site remains to be established, we have demonstrated that the hidden C_L site can be exposed on the whole immunoglobulin molecule. Intact immunoglobulin G, immunoglobulin A, and immunoglobulin D myeloma proteins and immunoglobulin M Waldenström macroglobulins (all type κ proteins) were treated with 8*M* urea, *p*H 8.9; subsequent immunochemical analyses, in which an antiserum specific for the hidden C_L antigenic site was used, revealed that the hidden site was exposed on the molecules belonging to the four different classes by treatment with the dissociating agent. The urea-treated immunoglobulins gave a precipitin reaction of identity with a C_L isolated from a κ chain. Similarly, in the presence of a dissociating agent (8*M* urea), pooled human immunoglobulin G (Cohn fraction II) was unfolded to expose the hidden C_L antigenic site.

The fact that the hidden C_L site can be exposed on the complete immunoglobulin molecule without cleavage of the molecule but simply by unfolding of the molecule generates specific questions regarding the function of the hidden antigenic site on the C_L. One might speculate that this site becomes exposed when the tertiary structure of the immunoglobulin molecule is altered as a result of reaction of the molecule with antigen, and that the site participates in the stabilization of the antibody molecule. The precise location of this hidden site in the C_L and the functional role of the site remain to be determined.

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Bubble Formation in Physical and Biological Systems: A Manifestation of Counterdiffusion in Composite Media

Abstract. *The counterdiffusion of gases across a composite layer can lead to supersaturation and development of bubbles within the layer. A physicochemical model has been derived to predict the extent of such supersaturation; experiments with inert liquid layers confirm predictions. These findings explain the evolution of cutaneous lesions observed in man during simulated deep-sea dives and the cutaneous lesions and intravascular bubbles experimentally induced in pigs by exchanging certain inert gases across the skin. The phenomena associated with counterdiffusion have widespread physical and biological implications.*

In an attempt to explain several puzzling physiological phenomena observed during simulated diving experiments, we have performed studies of gas counterdiffusion in composite layers. The theoretical analysis and experimental work which followed this line of inquiry have uncovered a variety of effects concerning gas exchange with potential applications in diverse fields, including hyperbaric and underwater physiology, anesthesiology, and membrane biophysics, and in studies of membrane separations and nucleation phenomena.

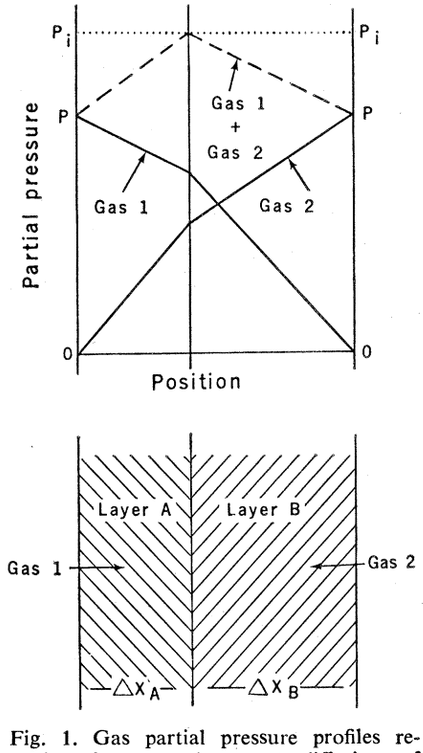

Fig. 1. Gas partial pressure profiles resulting from steady counterdiffusion of two inert gases through a two-layer composite of materials having different permeabilities for the two gases (see text).

The initial observations were made during a series of experiments carried out at the Institute for Environmental Medicine, University of Pennsylvania (Predictive Studies III) (*1*). Subjects in a helium-oxygen environment at a constant elevated pressure developed intense itching; gross, confluent maculopapular skin lesions (*2*); and an incapacitating vestibular derangement including vertigo, nausea, and nystagmus (*3*) within an hour after beginning to breathe a nitrogen-oxygen or neon-oxygen mixture through a mask or mouthpiece. Itching and skin lesions in man under related circumstances had been reported by investigators at Duke University (*4*). The skin manifestations could be prevented by covering exposed skin areas with a relatively impermeable suit ventilated with the same gas mixture that was being breathed.

The investigators at Duke had postulated osmotic gradients and the water flux produced by these gradients (*5*) as a causative mechanism for the skin lesions. We examined the counterdiffusion of two gaseous species through a two-layer structure and concluded (*6*) that, under the proper conditions, supersaturation with the attendant possibility of bubble development would exist in some region within the two-layer system (that is, the sum