would be interesting to conduct systematic surveys of patients with adult non-Hodgkin's lymphoma in various regions of the African continent, with an emphasis on clinical, pathologic, and immunopathologic features of the disease. A study of sera from African patients with known or suspected T-cell malignancies, including the acquired immune deficiency syndrome (AIDS) (*24*), would help to clarify the distribution of the HTLV family and the diseases associated with it, especially in view of the high HTLV-I antibody level in the Ugandan group, the occurrence of AIDS in neighboring Zaire (*25*), and the occurrence of Kaposi's sarcoma along the equatorial region of Africa with its highest prevalence in eastern Zaire and western Uganda (*26*).

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16. We expect that these and other types of indirect assays currently applied without a specific confirmatory step, including immunofluorescence assays that rely on the use of live or fixed HTLV-I–infected lymphocytes, would also be sensitive to such drastic elevations in IgG and could lead to overestimation of seropositivity in similar cases.
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Sulfation and Phosphorylation of the Neural Cell Adhesion Molecule, N-CAM

Abstract. *Embryonic chicken brain tissue cultured in media containing ^{35}S-labeled sulfate or ^{32}P-labeled phosphate incorporated ^{35}S or ^{32}P into the neural cell adhesion molecule (N-CAM). The ^{35}S label was located in asparagine-linked carbohydrates on both glycopeptides (molecular weights, 170,000 and 140,000) but not in the sialic acid. The ^{32}P label was detected in phosphoamino acids in the carboxyl-terminal third of both polypeptides, but the ratio of phosphoserine to phosphothreonine differed in the two species. The sulfated saccharides and phosphoamino acids may provide additional sites for functional control of N-CAM.*

The neural cell adhesion molecule (N-CAM) mediates neuron-neuron and certain nerve-muscle adhesions (*1*). The N-CAM from embryonic tissue is distinguished by its high sialic acid content (30 percent), much of which is present as polysialic acid (*2*, *3*). During development, the amount of sialic acid on N-CAM decreases to varying extents and at different rates in different brain regions (*4*). This conversion from embryonic to adult forms of the molecule appears to increase the rate of homophilic (N-CAM to N-CAM) binding (*5*). Most of the sialic

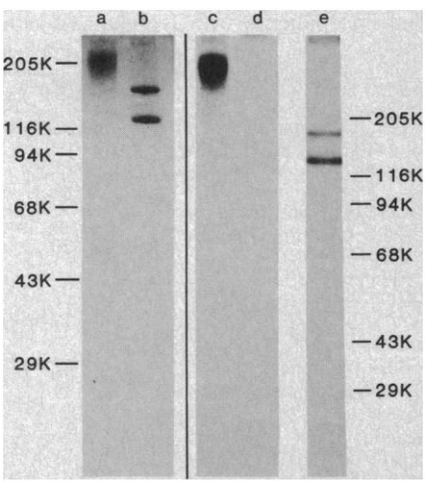

Fig. 1. Location of ^{35}S-labeled sulfate in N-CAM. ^{35}S-labeled N-CAM incubated with no enzyme (lanes a and c), endoglycosidase F (lanes b and d), or neuraminidase (lane e) and detected by Coomassie blue staining (lanes a and b) or fluorography (lanes c through e). Tissue from brains from 50 chicken embryos (7 to 9 days old) was labeled (*6*) in 40 ml of sulfate-free Eagle's minimum essential medium (EMEM) (*21*) containing 4 mCi of ^{35}S-labeled sulfate (Amersham), and N-CAM was isolated from clarified NP-40 extracts with monoclonal antibody anti-(N-CAM) 1 coupled to Sepharose CL-2B (*3*). (Lanes a through d) N-CAM was eluted from the immunoaffinity gel with 30 mM diethylamine and 1 mM EDTA, dialyzed, and lyophilized. Portions (25 μg) of N-CAM were boiled in SDS (1.43 percent), dithiothreitol (71 mM), and tris-HCl (1.4 mM, pH 7.4) for 3 minutes. Four volumes of NP-40 (1.6 percent), $NaPO_4$ (0.34M, pH 6.3), EDTA (0.16M), Trasylol (10^3 unit/ml), and phenylmethylsulfonyl fluoride (235 ng/ml) were added, and the samples were incubated (18 hours at 37°C) with 3.75 units of endoglycosidase F (lanes b and d) or without enzyme (lanes a and c); samples were resolved on a 7.5 to 15 percent acrylamide gradient gel. (Lane e) Sulfur-35–labeled N-CAM (10 μg) was treated with neuraminidase while still bound to the immunoaffinity support (*3*), eluted in sample buffer (*12*), and resolved on a 7.5 percent acrylamide gel. Standards given on the right apply only to lane e.

acid in N-CAM is found adjacent to the amino-terminal part of the protein, which contains the binding site (*6*), so its effect is probably indirect.

To analyze other structural features of N-CAM that could modulate its expression or function, we examined the incorporation of sulfate and phosphate groups into embryonic chicken N-CAM in vitro. Sulfation and phosphorylation of some sugar moieties in glycoproteins is known (*7*); however, phosphate is more commonly located on amino acids, particularly serine, threonine, or tyrosine, and often on such residues present on the cytoplasmic portions of transmembrane proteins (*8*).

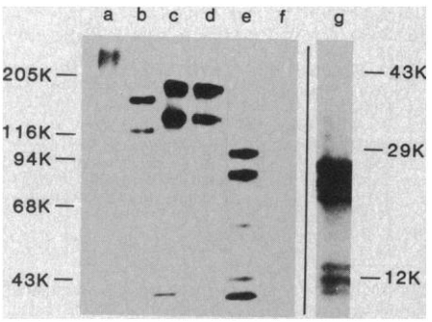

Fig. 2. Location of ^{32}P-labeled phosphate in N-CAM. Phosphorus-32–labeled N-CAM without (lane a) and with (lane b) endoglycosidase F treatment. Intact N-CAM (lanes c and d) and fragments of N-CAM released from membranes by *S. aureus* V8 protease (lanes e and f) were prepared from brain tissue incubated with ^{3}H-labeled leucine (lanes c and e) or with ^{32}P-labeled phosphate (lanes d and f) and were digested with neuraminidase. (Lane g) Peptides from treatment of ^{32}P-labeled N-CAM with CNBr. Samples containing ^{32}P were detected by autoradiography, and samples containing ^{3}H were detected by fluorography. (Lanes a and b) N-CAM was prepared as described (see legend to Fig. 1) with phosphate-deficient EMEM containing 4 mCi of ^{32}P-labeled phosphate (Amersham). Portions (10 μg) of N-CAM were incubated with 1.5 U of endoglycosidase F (see Fig. 1) or without enzyme and were resolved on a 7.5 percent acrylamide gel. (Lanes c through f) Brain tissue was incubated with either ^{32}P-labeled phosphate or ^{3}H-labeled leucine; cultures were harvested separately, and membranes were prepared from each (*3*). A portion (80 percent) of each preparation was resuspended in 1 ml of phosphate-buffered saline and digested with 100 μg of *S. aureus* V8 protease (Miles) for 30 minutes at 37°C. The released fragments were separated from the membranes by centrifugation. Intact N-CAM in NP-40 extracts of the untreated membranes and N-CAM fragments from the *S. aureus* V8 supernatants were immunoprecipitated, digested with neuraminidase, eluted, and electrophoresed (see Fig. 1). (Lane g) Phosphorus-32–labeled N-CAM was digested with endoglycosidase F (see Fig. 1), and NP-40 and SDS were removed from the sample (*22, 23*). The dialyzed, dried material was treated with 10 percent (weight to volume) CNBr in 70 percent formic acid for 2 hours at ambient temperature and then resolved on a 7.5 to 16 percent acrylamide gradient gel. Standards indicated on the right apply only to lane g.

Embryonic brain tissue incubated in media containing ^{35}S-labeled sulfate or ^{32}P-labeled phosphate incorporated label into embryonic N-CAM (Fig. 1, lanes a and c; Fig. 2, lane a), reaching maximum concentrations in 15 hours. The stoichiometry of phosphate and sulfate in N-CAM could not be estimated by this method because of dilution by endogenous pools, but previous colorimetric assays for phosphate (*9*) suggested that there were about three phosphate groups per N-CAM polypeptide (*3*) [average molecular weight, 145,000 (145K)]. D2, a rodent protein related to N-CAM, has also been observed to incorporate ^{32}P from ^{32}P-labeled phosphate (*10*).

Initial determination of the location of the ^{35}S and ^{32}P incorporated into N-CAM was obtained by digestion with neuraminidase to remove the sialic acid or with endoglycosidase F to remove all asparagine-linked oligosaccharides (*11*). Neuraminidase changed the migration of N-CAM on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (*12*) from a diffuse zone to two discrete components with molecular weights of 170K and 140K (*3*). All of these forms were labeled with ^{35}S and ^{32}P (Fig. 1, lanes c and e, and Fig. 2, lanes a and d), and no ^{35}S or ^{32}P was detected in the released material. Endoglycosidase F further decreased the apparent molecular weight of N-CAM; the resultant 160K and 130K peptides (Fig. 1, lane b, and Fig. 2, lane b) were similar in migration to N-CAM synthesized by cells treated with tunicamycin (*6*), suggesting that the enzyme released all asparagine-linked oligosaccharides. Endoglycosidase F removed more than 98 percent of the ^{35}S (Fig. 1, lane d) from N-CAM but less than 12 percent of the ^{32}P (Fig. 2, lane b). These data imply that the ^{35}S in N-CAM was present in carbohydrate residues other than sialic acid of the asparagine-linked oligosaccharides, while the ^{32}P was bound directly to amino acids. In accord with the notion that the ^{35}S is on carbohydrates, experiments have indicated that the CNBr fragment of N-CAM that contains the major oligosaccharides (*13*) also contained most of the ^{35}S incorporated into the molecule. Other studies have shown that polysialosyl glycopeptides from whole brain contain *O*-sulfate residues (*14*).

The ^{32}P in N-CAM was detected in phosphoserine and phosphothreonine in acid hydrolysates (*15*) of both the 160K and 130K polypeptides (Fig. 3). Elution of the phosphoamino acids from the electrophoretograms and scintillation counting indicated that the ratio of phosphoserine to phosphothreonine in the 160K component was greater than one, while the ratio in the 130K form was less than one (Fig. 3).

To determine further the location of the phosphate, we examined the fragments of N-CAM released from brain membranes (*3*) by *Staphylococcus aureus* V8 protease. In earlier studies (*3, 6*) a series of N-CAM fragments was identified in such digests, including one 108K peptide (Fr2), which contains the amino terminus, the N-CAM binding region, and the polysialic acid attachment site or sites. In our experiments, this series of N-CAM fragments was generated from digests of membrane preparations from tissue cultured with ^{3}H-labeled leucine or ^{32}P-labeled phosphate. The intact N-CAM molecules incorporated both isotopes; however, the fragments derived from them contained ^{3}H but not ^{32}P (Fig. 2, lanes c to f), indicating that the phosphate groups in N-CAM are in that portion of the polypeptide which is carboxyl-terminal to Fr2 and raising the possibility that they are located on a cytoplasmic domain. However, protein kinases that phosphorylate external portions of proteins have been described (*16*).

More precise location of the phos-

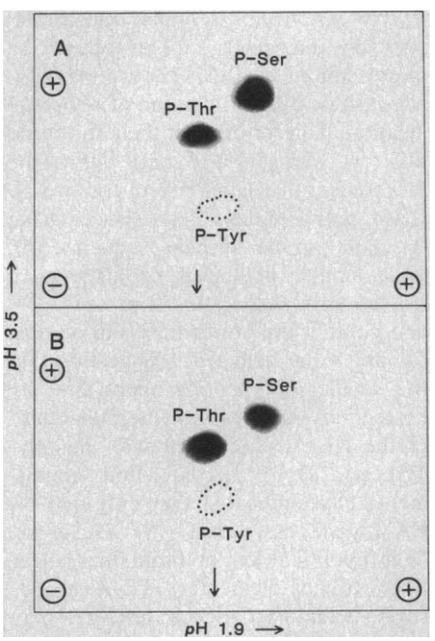

Fig. 3. Analysis of N-CAM phosphoamino acids. Phosphoamino acids from the 160K (A) and 130K (B) N-CAM polypeptides were separated by two-dimensional thin-layer electrophoresis and detected by autoradiography. Phosphorus-32–labeled N-CAM was purified and digested with endoglycosidase F (see Fig. 1). The 160K and 130K products were separated on a 7.5 percent acrylamide gel, excised, eluted, hydrolyzed, and analyzed (*14*). Positions of phosphoamino acid standards electrophoresed with the samples and detected by ninhydrin staining are indicated. Arrows (↓) indicate origin.

phate-containing region of the molecule was complicated by the observation that CNBr digestion of ^{32}P-labeled N-CAM (Fig. 2, lane g) yielded at least six phosphorylated fragments distributed into two zones (19K and 10K) on SDS-PAGE; they did not correspond to any previously identified CNBr fragments. They could have resulted from partial acid hydrolysis of aspartyl-prolyl peptide bonds, but we have no evidence for such bonds in N-CAM; after treatment with 70 percent formic acid without CNBr, the N-CAM polypeptides were not soluble in SDS-PAGE sample buffer (*12*) even after boiling. These peptides probably arise from another source, such as multiple phosphorylation of two regions in one or both N-CAM polypeptides; one zone of three components might be derived from the smaller polypeptide (130K) and the other from the larger one (160K). Differences in the phosphorylation of the N-CAM polypeptides is suggested by their different ratios of phosphoserine to phosphothreonine (Fig. 3) and by preliminary *S. aureus* V8 protease mapping (not shown).

Defining the structural features that distinguish the N-CAM polypeptides is important for establishing the organization of the molecule, its interaction with the cell membrane, and the regulation of its expression. For example, transformation by oncogenic viruses alters the expression of N-CAM (*17*) and may also affect the extent or location of phosphorylation. A description of their structural differences might also help define the mechanism that gives rise to the two N-CAM polypeptides. Both species have the same amino terminal sequence (*6*), yield similar unlabeled peptides when treated with *S. aureus* V8 protease (*3*), and contain the attachment site or sites for polysialic acid (*13*), suggesting that the smaller polypeptide might arise by proteolysis in the carboxyl terminal third of the larger. Consistent with this hypothesis, DNA hybridization experiments have indicated that only one N-CAM gene may exist (*18*). However, filter hybridization of brain messenger RNA to complementary DNA probes suggests that two N-CAM mRNA's may be produced (*18*). The synthesis of N-CAM could thus resemble that of fibronectin, which has similar polypeptides resulting from differential processing of a single heterogeneous nuclear RNA (*19*). Regardless of the origin of the differences in the N-CAM polypeptides, the phosphoamino acids may serve as distinguishing markers.

During neuronal development, N-CAM expression, distribution, and binding are probably controlled by several mechanisms (*1*). Alterations in the sialic acid content of N-CAM (*4*) and the amount of N-CAM on membranes occur in vivo (*20*); both have striking effects on N-CAM binding in vitro (*5*). The relative prevalence of the 170K and 140K forms of N-CAM differs among regions of the central nervous system (*1, 4*), but the function of this variation remains to be defined. The differences in labeling presented raise additional possibilities for functional control. Sulfation and phosphorylation probably affect N-CAM function indirectly, perhaps participating in the regulation of its prevalence or distribution on the cell surface, its degree of sialylation, or the stability of intermediate forms produced during intracellular or extracellular processing.

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Tactics of Acclimation: Morphological Changes of Sponges in an Unpredictable Environment

Abstract. *Reciprocal transplants of genetically identical fragments of intertidal sponges between environments of high and low wave action exhibit great variability in the timing of their responses to environmental change. Sponges quickly begin production of stiffer and stronger tissues in high wave energy environments but delay formation of new, weak tissues in calm habitats. This may be due to the risks of forming wave-intolerant tissue in a temporally variable, unpredictable environment. These results suggest that the evolution of acclimatory control is linked to environmental predictability and concomitantly to risks of acclimatory errors.*

Although much is known of mechanisms of physiological acclimation and their environmental control (*1–3*), very little is known of the internal developmental changes that lead to acclimatory responses (*2, 4–6*). By transplanting genetically identical sponge fragments into a range of environments and monitoring the morphology of new tissues produced, I have recorded the rates of response to benign versus stressful environments. Wave-tolerant morphologies are quickly produced in transplants into stressful environments. However, acclimation to wave intolerance is delayed in transplants into benign environments. Separate experiments show that risks of maintaining inappropriate morphologies are asymmetric; the morphology characteristic of benign environments is intolerant of heavy wave action and is at risk because of environmental uncertainty. Thus, asymmetric rates of response to the environment parallel asymmetric risks. This suggests that the process of acclimation to and away from stress tolerance is coupled to the relative risks of stress tolerant and intolerant states.

In the demosponge *Halichondria panicea*, colony morphology varies with