the serums of single rabbits. The assays were performed under carefully controlled conditions; an analysis of an amino acid standard was alternated with each analysis of an antibody hydrolysate and a single pipette was used for the application of the samples on the long and short columns of the analyzer. The average recoveries obtained for each antibody after 20 hours of acid hydrolysis and the calculated errors of an individual determination are given in Table 1. The data have been expressed as moles of residue per mole of antibody and normalized to a leucine content of 89. The value of 89 represents the average recovery of leucine per mole of antibody as calculated from nitrogen analyses of the antibody hydrolysates, a nitrogen content of 16 percent and a molecular weight of 160,000 (*1*).

The average amino acid composition of arsonic antibody (column 1) was identical to that previously obtained when arsonic and ammonium (*4*) antibodies were prepared in the same rabbit (*1*). These results permitted the comparison of the lac antibody with the previously published data since they demonstrated that the amino acid composition of any one antibody was not detectably affected by the simultaneous production of another antibody. Furthermore, these results provided additional evidence that the amino acid compositions were independent of the γ-globulin allotypy since the antibody preparations used were isolated from both heterozygous and homozygous animals.

The average values for the lac antibody (column 2) were in most cases strikingly similar to those of its arsonic control. However, a few small but significant differences appeared. The lac antibody was characterized by a higher aspartic acid content while the arsonic antibody had a higher tyrosine content. These differences were shown by standard statistical methods to have a 99.9 percent probability of being outside experimental error. In addition, the serine value was 3 percent lower in the lac antibody, but whether this recovery reflected a lower initial content or a slightly increased lability to acid hydrolysis could not be determined without further measurements of the rate of serine destruction.

The standard deviations of an individual determination listed in columns 3 and 4 were very similar for both antibodies despite the fact that the arsonic antibody was always prepared against a hapten-bovine γ-globulin complex whereas the lac antibody was prepared against hapten conjugated either to bovine γ-globulin or to bovine serum albumin. Thus, the observed differences in amino acid compositions could not be related to the protein carrier used in the immunization.

Since ammonium antibody has been shown to differ from arsonic antibody in its aspartic acid, arginine, leucine, and isoleucine content (*1*), while lac antibody differs only in its aspartic acid and tyrosine contents, these combined data established that each of the three antibodies has a unique amino acid composition. It was conceivable that differences in amino acid content of two antibodies could be explained by the heterogeneity of γ-globulin especially since two pools of globulin which differ slightly in their amide and charged amino acid content have been found in studies of the univalent fragments liberated by papain digestion (*8*). This hypothesis becomes much more improbable with the demonstration of a third unique antibody composition because it would require not only the existence of three different globulin pools in all the animals used, but also the distribution of one antibody in each pool. A more probable explanation is that the observed differences in amino acid composition are directly related to the one property which distinguishes these antibodies from each other, the specificity of their reaction with respective antigen.

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Nerve Fibers and Terminals: Electron Microscopy after Nauta Staining

Abstract. *Sections of cat spinal cord and rat mammillary body in which degenerating nerve fibers were stained by the Nauta silver method have been examined with the electron microscope. Silver granules were present in the axoplasm of some myelinated fibers and in some of the axon terminals.*

The Nauta method stains degenerating nerve fibers selectively, and shows the localized swellings and discontinuities that occur along the degenerating fibers (Fig. 1). The method has been widely used for tracing fiber pathways in the central nervous system, but there is disagreement about its suitability for studying synaptic relationships (*1*, *2*). Whereas the Glees silver method stains some synaptic boutons as readily recognizable rings or clubs, the Nauta method either does not stain the boutons at all or stains them as swellings not clearly distinguishable from the swellings that occur all along the course of degenerating axons (Fig. 1).

This study was undertaken to determine whether the Nauta method can stain terminal boutons and also to find

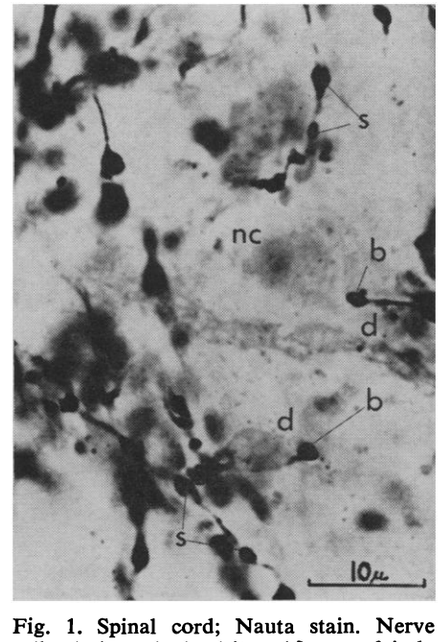

Fig. 1. Spinal cord; Nauta stain. Nerve cells (*nc*) and dendrites (*d*) are faintly stained. The degenerating fibers are dark. Two appear to end as boutons (*b*), but these boutons cannot be easily distinguished from the swellings (*s*) that occur along the course of the degenerating fibers.

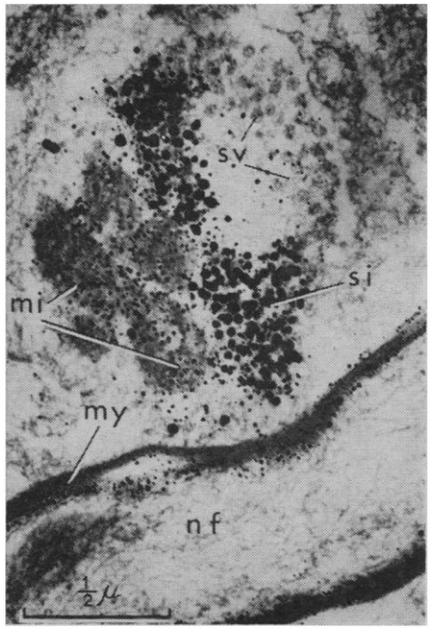

Fig. 2. Spinal cord. An axon terminal that has been stained by the Nauta method. The terminal can be identified by the synaptic vesicles (*sv*) and by the clump of mitochondria (*mi*), and contains silver granules (*si*). At the bottom is a myelinated fiber that shows a few silver granules and no staining of the neurofilaments (*nf*); myelin, *my*.

out whether it would be possible to mark degenerating terminals with silver for subsequent study of synaptic relations by electron microscopy.

Dorsal roots (L_6 –S_1) in a cat were sectioned central to the ganglia and 5 days after the operation the animal was perfused with cold Ringers solution and then by formol sucrose. Both solutions were buffered to *p*H 7.0 to 7.2. Sections between 50 and 150 μ thick were cut from the unfrozen spinal

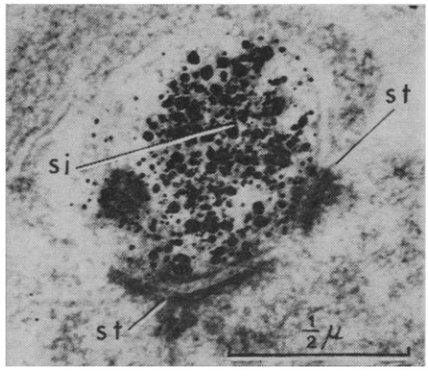

Fig. 3. Spinal cord. An axon terminal that contains silver granules (*si*) and that can be identified by the synaptic thickenings (*st*).

cord and treated by a Nauta method (*3*). The sections were dehydrated, stained in phosphotungstic acid, and embedded in a layer of araldite about 2 mm thick. Sections that showed good impregnation of degenerating fibers and a reasonable suppression of normal fibers were then sectioned for study with the electron microscope. The mammillary bodies from a rat that had had the fornix cut 7 days before death were similarly treated.

Although the preservation of the tissue was poor it was possible to recognize myelin, neurofilaments, synaptic vesicles, and the membrane thickenings that characterize synaptic contacts (Figs. 2–4). Coarse silver granules were seen in the axoplasm of some myelinated fibers (Fig. 4) and in some axon terminals (Figs. 2 and 3) in all the preparations. Many axon terminals, identifiable by vesicles and thickened contact regions, were free of silver. The silver granules were often localized within a portion of the axons (Fig. 2) where the mitochondria and the synaptic vesicles of the terminal, and the neurofilaments of the myelinated fiber are unstained. It has also been possible to see unstained neurofilamentous rings in several terminals. So far we have been unable to determine what specific part of the axoplasm is "Nauta positive" in these preparations.

The terminals that were stained varied in size from 0.5 to 2 to 3 μ in diameter. It would thus seem that both the Glees and the Nauta methods can stain degenerating terminals. The Glees method stains primarily the neurofilaments of the endings (*4*) and, because these filaments are often grouped to form characteristic rings or bulbs, the endings can be recognized readily with the light microscope. The Nauta method stains another, at present unidentified component of the axoplasm, which generally shows no characteristic morphology in the axon terminals. The Nauta sections can, however, be usefully studied with the electron microscope. Such studies can show the localization of terminal as distinct from preterminal degeneration. Also, since the synaptic membrane thickenings are relatively well preserved, the present method may prove useful for studying the types of synaptic contact that are made by particular groups of fibers.

In some parts of the nervous system the neurofilaments of the terminals increase markedly during degeneration,

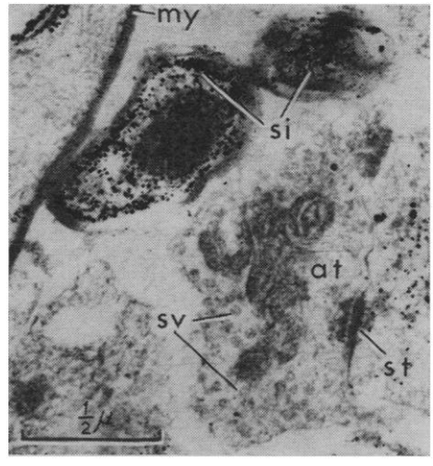

Fig. 4. Spinal cord. An axon terminal that is free of silver (*at*) lies adjacent to two small myelinated fibers which both contain silver granules (*si*).

while in other parts there are no neurofilaments in the terminals at any stage of degeneration (*4*, *5*). In the first-mentioned case the terminals can be stained by the Glees method, while in the other case they cannot be so stained. Similarly, it appears that terminals in some parts of the nervous system can be stained by the Nauta method while in other parts they cannot. At present it seems that the Nauta method fails to stain the terminals that show the most marked Glees staining (*2*). It remains to be determined whether there are any terminals that can be stained by both the Nauta and the Glees methods, and also whether there are terminals that cannot be stained by either method.

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