A and A′, or both, in the tetramers. There would appear to be no advantage in the hybrid's having all types of subunits.

The tissue specificity is particularly striking in extracts of eyes assayed for lactate dehydrogenase activity. There are at least two rapidly migrating isozymes of this enzyme in trout eyes, and they appear to be present as relatively major components (Fig. 3, pattern A). This observation is in agreement with that of Markert and Faulhauber (*9*) that a very negatively charged LDH molecule is present in eye extracts from approximately 30 species of fish. These investigators suggest that there is an independent genetic origin for these isozymes relative to the other forms of LDH in the fish. Such a conclusion is supported by the outcome of the dissociation-recombination experiment presented here, when isozymes which must contain other than an A or B subunit are formed (Fig. 3, pattern B).

Thus the molecular heterogeneity of lactate dehydrogenase in fish is relatively complex compared with the LDH isozyme complement of the more intensively investigated mammalian system (*9, 10*). Some of this complexity is clarified by the unequivocal demonstration here that in the hybrid fish heterozygosity at one genetic locus and co-dominance of alleles at this locus result in the production of three polypeptide subunits which are utilized in the synthesis of homo- and heteropolymers of a single protein. In view of these data it is necessary that I withdraw my original interpretation of the isozyme pattern in trout as representing the activity of three non-allelic genetic loci (*1*) in favor of the alternative explanation provided here. However, the original premise that three types of subunits participate in the synthesis of trout lactate dehydrogenase remains valid.

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7. An analysis, completed after submission of this report, of LDH patterns in 60 speckled trout embryos from the Gaspé Hatchery reveals that a mutant *b* gene occurs in this population. Approximately 50 percent of the animals have the five isozymes described in this report, 35 percent show the nine-isozyme pattern described previously (*1*), and 15 percent have five isozymes with an electrophoretic distribution suggesting a new B monomer and therefore a mutant *b* gene. Thus the three LDH genotypes in this population are, *bbaa*, *b'b'aa*, and *b'baa*.
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Electron Microscopy: Two Major Synaptic Types on Spinal Motoneurons

Abstract. *Two major types of synaptic bulbs are defined on the motoneuron surface of the monkey, on the basis of content of agranular "synaptic" vesicles of two distinct kinds. Both types are present on dendritic as well as perikaryal surface. Because of the approximately equal numbers, the hypothesis that one type is excitatory and the other inhibitory naturally arises.*

In recent years the additional complexities of synaptic structure revealed by the electron microscope have fortunately coincided with the development of fundamental new evidence concerning synaptic function. The evidence for the existence of inhibitory as well as excitatory synapses, of electrical as well as chemical synaptic transmission, of a variety of possible transmitter substances, and of differential electrical properties of postsynaptic elements, whether dendrites, somata, or axons, now offers the histologist an arena of enhanced opportunity for meaningful discovery of functional anatomical relationships.

Whereas important evidence has been obtained which links structural attributes to electrotonic as contrasted to chemical synaptic transmission (*1*), the relationship of structural differentiation to excitatory or inhibitory function is much less clear. A promising lead, based upon differences of the synaptolemma (junctional interface) on dendrites as contrasted to perikaryal surface in the cerebral cortex (*2*), has not subsequently yielded a clear picture of the significance of such differences. Moreover, the distribution of granular synaptic vesicles is not such as to suggest a general correlation with excitatory or inhibitory functions (*3*). Our findings for the spinal cord are therefore of special interest; they reveal a differentiation of two major types of synaptic bulbs on the basis of the morphology of agranular vesicles.

It is probable that the fixative used (2.5 percent buffered glutaraldehyde) made possible a clearer differentiation of types of synaptic bulbs than we obtained in earlier studies with formaldehyde fixation (*4*), but other details of preparation, including hardening with osmium tetroxide, araldite embedding, and staining with lead hydroxide, were the same. Synaptic boutons were characterized according to type of vesicle location on postsynaptic surface (soma, dendrite, or dendritic spine), mode of origin (myelinated or unmyelinated telodendria, or node of Ranvier), size, and relation to subsynaptic cistern or Nissl body. Two major types, well shown in Fig. 1, and two additional types, of infrequent occurrence, were characterized in the motoneuron neuropil. The two major types were found not only on motoneuron dendritic and perikaryal surfaces but also on smaller neurons of the intermediate zone of the anterior gray column. In all locations they tend to be almost equally distributed, but there is a tendency for clustering of each type in any particular location.

One type, which tends to be slightly more numerous, contains agranular vesicles with circular profiles of about 250 to 400 Å, hereafter referred to as S vesicles. The other type contains flattened, elongated, agranular vesicles which may be 100 to 200 Å wide and 300 to 600 Å long (F vesicles). The "F" boutons may contain an admixture of S vesicles, whereas "S" boutons do

not contain F vesicles. Of 1000 synaptic bulbs categorized, 45 percent were of type S and 39 percent were of type F.

The mixture of the two types of synaptic bulbs on motoneuron dendrites and perikarya is in contrast to the interesting finding that dendritic spines were surmounted in each observed case by a type F synaptic bulb. Because of the small number of spines in the motoneuron neuropil (seven), it seems possible that the dendrites were those of interneurons rather than of motoneurons, although the latter cannot be excluded. In a few instances the dendrite carrying the spine bore both the type F synaptic bulb on the spine, as well as type S synaptic bulbs elsewhere. No characteristic location was found on the motoneuron surface where only type S synaptic bulbs were present, although axon hillocks were not identified in our material. It is of interest, however, that nine of ten synaptic bulbs which arose at nodes of Ranvier in the neuropil were of type S, and a similar relation was observed among synaptic bulbs which formed the direct terminations of myelinated axons.

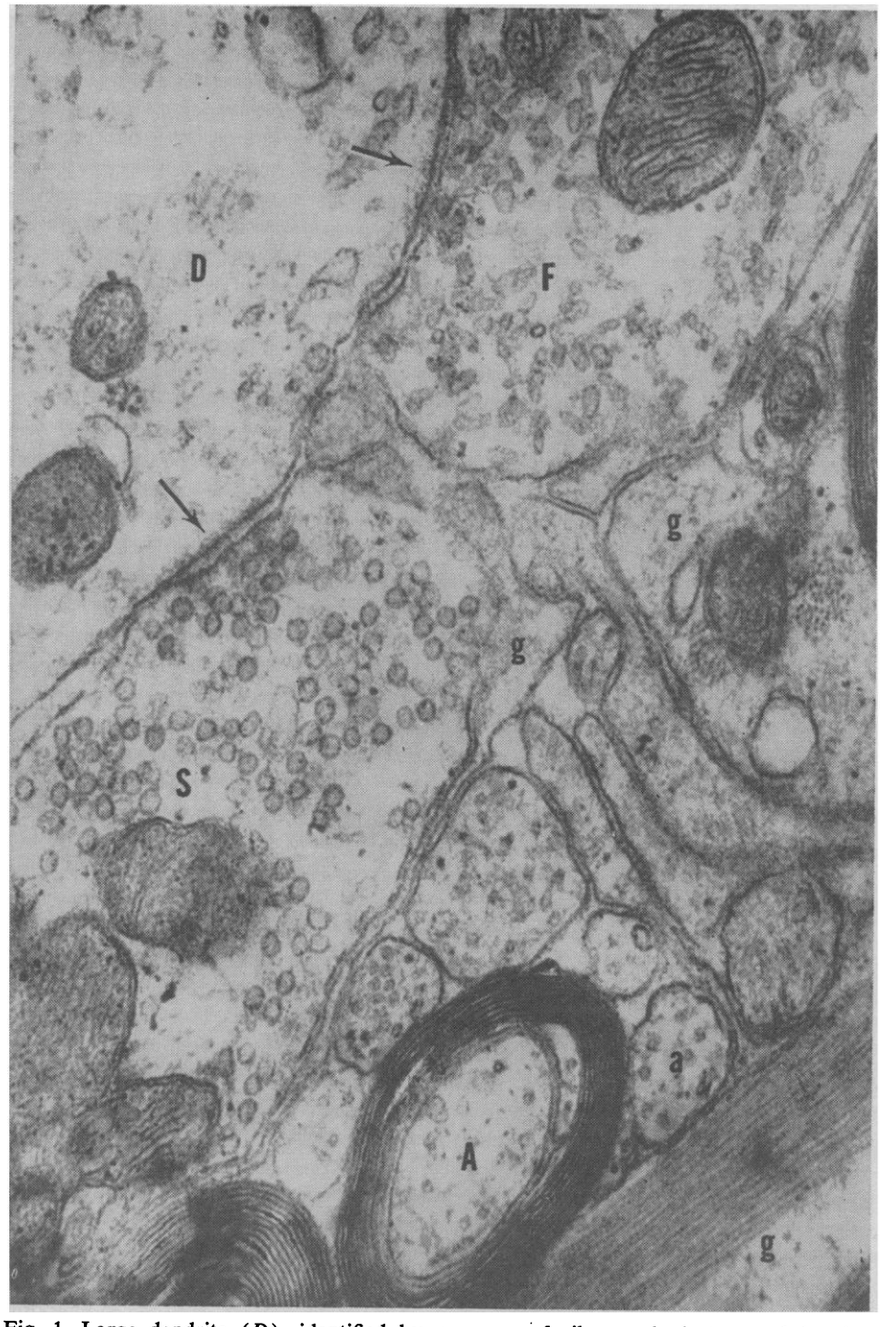

Fig. 1. Large dendrite (*D*), identified by presence of ribosomal cluster, and bearing two types of synaptic bulbs (*S* and *F*) side by side. Arrows indicate synaptolemmal attachment sites; *A*, myelinated axon; *a*, unmyelinated axon or axon telodendrion; *g*, glial process (× 78,000).

The finding of two dominant types of synaptic bulbs on motoneurons might be interpreted as a result of functional variation of vesicle form in what may conceivably be a single population of bulbs. Yet the observation of similar types on other neurons in the spinal cord suggests a deeper significance of the two types; this suggestion is reinforced by a small number of data pointing to dominance of one or the other type in certain locations (dendritic spines and nodal synaptic bulbs). The mode of fixation, while allowing a clear discrimination between the two types, is not a critical factor, for the distinction is present, although less clearly so, in animals perfused with Regaud's solution rather than glutaraldehyde.

It would be tempting to speculate that the two major types of boutons on spinal cord motoneurons represent the expected functional dichotomy of excitatory and inhibitory synapses. The approximately equal numbers suggest such an interpretation, although it is obvious that we must await experimental evidence. Although small regions of the neuron surface sometimes appeared to show aggregation of one or the other type of synaptic bulb, the presence of both major types on dendrites and somata indicates that, if the two types represent inputs of opposite sign, then excitatory and inhibitory "spots" would not be exclusively segregated on either dendrites or somata. In the event that the significance of the two types of synaptic knobs turns out to be unrelated to the functions of excitation and inhibition, further analysis of the distribution and properties of the two types would still seem to promise results of functional importance.

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