rounded up fully during treatment, with detachment and absorption of their processes; the small daughter cells were also lost during fixation. In the present study, only clearly identifiable binucleate neurons (remaining on the fixed culture slides) were included in the assay of mitotic activity; the percentages of mitotic neurons given herein (Table 1) are thus minimums. The true percentages are possibly double those indicated, as estimated from the periodic observations. A similar situation existed in the case of the DNA synthesis determinations; detachment and loss of activated cells also occurred in substantial numbers, but with somewhat less frequency than in the mitotic cells, due perhaps to the shorter treatment period. The possibility that the binucleate cells observed arose from induced or spontaneous fusion of adjacent neurons, rather than from nuclear division, was precluded by use of cultures in which the individual test neurons were well dispersed.

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4 September 1975; revised 31 October 1975

Neural Transport of Tetanus Toxin

In figure 2 of their report, Price *et al.* (*1*) suggest that ^{125}I-labeled tetanus toxin is taken up at neuromuscular junctions and carried in motor axons by retrograde axonal transport to the central nervous system. With the nerves not crushed but intact one should then be able to demonstrate intraaxonal radioactivity at the level of the ventral roots. We succeeded in doing so (*2, 3*). Moreover, in intact (noncrushed) peripheral nerves we (*2, 3*), as well as Gardner and Fedinec (*4*), found a high amount of tetanus toxin in the epineurium. If the epineurium is opened, tetanus toxin leaks out (*5*). Transport of ^{125}I-labeled tetanus toxin also in sensory axons cannot be ruled out: after intramuscular injection of labeled toxin we (*3*) and Stöckel *et al.* (*6*) found radioactivity in cell bodies of pseudounipolar cells of the spinal ganglia. In the spinal cord, the perikarya of some motoneurons were heavily labeled (*2, 3, 6, 7*). Whether radioactivity found in the vicinity of the perikarya has been released from the motoneurons into the neuropil is still an open question.

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14 July 1975

Price *et al.* (*1*) show the presence of a ^{125}I-labeled product of tetanus toxin within the axoplasm of motor nerves supplying muscles into which the labeled material has been injected. The authors "think it likely that tetanospasmin can reach the CNS [central nervous system] by retrograde intraaxonal transport, but we cannot exclude some contribution by the circulatory system." Thus they offer evidence in support of the hypothesis first advanced by Marie and Morax (*2*) that tetanus toxin ascends from the wound site via the motor nerves to the CNS where it produces the signs of tetanus intoxication. This hypothesis was proposed to explain the clinical phenomenon of local tetanus. Briefly stated, this is the development of muscle stiffness localized solely to the site of a wound containing toxin. Because Marie and Morax did not consider that the toxin might also act on the striated muscle, it was necessary to postulate intraneural centripetal spread to the corresponding innervating neurons to explain the specificity and localization of the initial local tetanus. It was recognized that if the naturally acquired or experimental dose of toxin was large enough, generalized tetanus, involving all the striated musculature, would follow the local tetanus.

In their emphasis on demonstrating intraaxonal ascent of the toxin and thus finally confirming the Marie-Morax hypothesis, Price and associates ignore the evidence indicating that the centripetal theory does not account for the principal features of tetanus intoxication. First, while the authors do not compare the toxicity of their preparations before and after iodination by testing animals via the same route of injection, their data indicate that the iodinated product is less toxic than the original material. Despite this, they provide no evidence to show that the radioactive material observed within axons is labeled active tetanus toxin rather than largely nontoxic labeled protein following a pathway common to many proteins. Several investigators using various methods have shown that active (toxic) tetanus toxin has such a high affinity for neural membranes that it would be expected to be immobilized within the terminal axons (*3*). Also in direct contrast with the work of others is the absence of label in the perineural space (*4*). Second, the authors give no quantitative data on recovery of the ^{125}I-labeled toxin; that is, how much toxin was actually found within the nerve at the time of sampling and how much was bound elsewhere either nonspecifically (in such organs as spleen and kidney) or specifically within the CNS? Was any attempt made to localize the toxin in those animals that had been killed with intraperitoneal doses of radioactive toxin and how did this distribution compare with the distribution of the toxin in the animals in which the toxin was injected intramuscularly before nerve crush? It is also important to consider the time intervals following intramuscular injection of the toxin because if a dose sufficient to produce intoxication is absorbed via lymphatics and blood vessels before the "hours later" of table 1 of (*1*), it is irrelevant whether labeled active or inactive toxin can be demonstrated in the axoplasm after this time. The lack of control data bearing on these points leads us

to conclude that while an iodinated product of tetanus toxin does indeed, like horseradish peroxidase and albumin, pass into and ascend in the terminal portions of motor axons, Price *et al.* (*1*) have not demonstrated the ascent of active toxin or that such ascent, if it occurs, is a principal route of entry of the toxin.

Since the work of Marie and Morax (*2*), direct effects of tetanus toxin on striated muscle have been demonstrated by many workers (*5*) and the effectiveness of circulating tetanus antibodies in preventing the disease has been a major contribution to preventive medicine. The large amount of experimental data does not yet offer a complete explanation of local tetanus but certainly does not leave the Marie-Morax hypothesis as the only possibility. We have offered the view that a combination of local changes in striated muscle together with CNS disinhibition is a sufficient mechanism for local tetanus (*5*). There is less controversy concerning generalized tetanus; most investigators agree that the toxin reaches the CNS via the circulation (*6*). As a more general point, we think the route of entry of tetanus toxin is a less rewarding topic of study than are the mechanism of toxin binding to its site of action and the nature of the lesion produced by so few molecules that have such a profound effect on the neuromuscular system.

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14 July 1975; revised 14 October 1975

As indicated by the comment of Wellhöner *et al.*, studies by Erdmann *et al.* (*1*) and Stöckel *et al.* (*2*), now published, have confirmed and extended our findings (*3*). Their studies showed that intramuscularly injected [^{125}I]tetanospasmin appeared in axons of the ventral roots. The rate of retrograde axonal transport was 7.5 mm/hour (*2*), and subsequently the radioactivity appeared in motor neurons (*1, 2*). Freezing (*1*) and colchicine treatment (*2*), procedures that block axonal transport, interfered with the migration of toxin. After freezing, radioactivity accumulated distal to the lesion (roots) and was reduced in the spinal cord (*1*). Although some label was present in dorsal root ganglia, the dorsal roots and dorsal horn showed no specific label (*2*).

These studies in part answer some of the questions raised by Zacks and Sheff. Our experiments (*3*) were specifically designed to show whether or not tetanus toxin was carried by retrograde axonal transport. In our studies, as in the investigation of Erdmann *et al.* (*1*) and Stöckel *et al.* (*2*), ^{125}I-labeled toxin was clearly present within axons. While it was not proved that the small amount of toxin within axons was active, several lines of evidence are against the concept that the accumulated radioactivity represents degradation products of toxin: (i) electrophoresis of our ^{125}I-labeled toxin showed one major protein component; (ii) this labeled toxin produced tetanus in rodents; (iii) by sodium dodecyl sulfate (SDS) gel filtration and SDS electrophoresis, the labeled material from spinal cords poisoned in vivo was indistinguishable from ^{125}I-labeled toxin added to homogenates already prepared (*4*); (iv) free ^{125}I was not present (*4*); and (v) active toxin has been recovered from peripheral nerves and roots (*5*). This indirect evidence is consistent with the concept that active toxin is being transported.

Zacks and Sheff (*6*) thought it unlikely that toxin was carried centripetally within nerve fibers. Their evidence consisted of an inability to demonstrate fluorescein-labeled toxin or peroxidase-labeled antitoxin within nerve terminals or axons. We do not have an explanation for their inability to show retrograde transport of toxin. We agree that the toxin does have an affinity for neural membranes; however, attachment of a substance to neural membranes does not necessarily mean that the material is immobilized. Neural membranes are probably both recycled (*7*) and transported retrograde within axons (*8*). The studies of Stöckel *et al.* (*2*) suggest that uptake of certain substances may depend on specific properties of neural membranes, but that the process of retrograde transport may be nonspecific. A possible mechanism of toxin transport may begin with the binding of toxin to some neurons at their exposed membranes (such as nerve terminals). The toxin could then be internalized during membrane recycling and, once within the nerve terminal, it could be loaded onto transport systems destined for the nerve cell body. Recent light and electron microscopic studies in our laboratory have shown that the toxin is taken up at nerve terminals and transported retrograde in association with membranous organelles (*9*).

Our initial study was confined to an examination of the intraaxonal pathway and did not address the role of the vascular spread of toxin or the pathogenesis of generalized tetanus. These remain open questions, but present evidence does not exclude an axonal route of entry in generalized tetanus. Tetanospasmin, with a molecular weight of approximately 130,000, reaches the bloodstream rapidly but enters the brain slowly. In one autoradiographic study (*2*), heavy label was present over blood vessels but there was no penetration of labeled toxin into the surrounding tissue, which suggests that the toxin is prevented from entering the central nervous system by the tight junctions of the blood-brain barrier. In addition, we have recently obtained evidence that systemically administered toxin can be carried centripetally by retrograde axonal transport (*10*).

Finally, we would suggest that the mechanism by which both endogenous and exogenous macromolecules are transported in the nervous system is a rewarding topic of study. We think that retrograde transport provides a route of entry for biologically active substances (for instance, nerve growth factor) and may be a pathway for entry of etiologic agents (for instance, viruses) in certain diseases of the central nervous system (*11*).

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29 December 1975