Case report

Vacuolation and chromatolysis of lower motoneurons in tetanus

A case report and review of the literature

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In recent years, the pathophysiological action of tetanus toxin has been extensively studied by many investigators.[1, 2] The role of the toxin in decreasing inhibitory input to alpha motoneurons is well known. Morphological alterations in neurons in both clinical and experimental tetanus have not been consistently described. Indeed, several authors simply state that no changes are found. There are, however, reports of nuclear capping, and either chromophobia or chromophilia[3] as well as central or perinuclear chromatolysis in motoneurons[4, 5] in tetanus. The finding of striking concurrence of central chromatolysis and vacuolation confined to motoneurons in a case of human tetanus prompts this report and a review of the literature.

Case report

A 58-year-old black man was admitted because of inability to swallow. The night before admission, he choked while trying to drink water; he also noted pain and stiffness in the neck and back. He was diabetic and took 40 units of insulin each morning. He had had an ulcer on his left heel for the preceding 10 months, but there was no history of recent wounds. He had not had a tetanus immunization for "many years." Chronic ulcers on the right leg had necessitated an above-the-knee amputation 1½ years before. The patient also had been taking phenytoin (Dilantin) and phenobarbital for a

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Supported in part by a grant from the Muscular Dystrophy Association.

seizure disorder, which apparently followed a stroke for which he had had a carotid bypass.

The patient initially went to the emergency room of a local hospital and was found to have risus sardonicus, opisthotonus, and dysphagia; the clinical impression was early tetanus. He was transferred to West Virginia Medical Center.

Physical examination revealed marked opisthotonus and masseter muscle spasm to the degree that the mouth could not be opened. The patient was moderately irritable and exhibited muscle spasms when touched. There was rigidity and increased tone of the paraspinal muscles. Examination of the heart, lungs, and abdomen was unremarkable. A 2 × 1-cm ulcer was found on the lateral aspect of the left heel; there was increased muscle tone in the left leg. The right leg demonstrated good stump healing with no ulceration. Cranial nerves were intact with pupils equal and reactive; there was no papilledema. Sensory examination revealed decreased light touch and vibration sense in the lower extremities; position sense was normal. Deep tendon reflexes were normal and symmetrical; there was no Babinski sign on the left.

Laboratory studies on admission revealed an elevated creatine phosphokinase (CPK) of 700 U/L (normal, ≤125 U/L) that rose to 1629 U/L five days after admission. The white blood cell count (WBC) was 11,200/mm^3 with a left shift; hemoglobin and hematocrit were 13.8 g/dl and 43%, respectively. Serum electrolytes were normal; glucose was 70 mg/dl. Lumbar puncture revealed 880 erythrocytes, 3 leukocytes, and a glucose of 65 with a peripheral glucose of 100. This was interpreted as a normal traumatic tap. Culture of the ulcer on the left heel grew *Clostridium tetani*. The patient was initially treated with 5000 units of tetanus immune globulin (Hyper-Tet) and intravenous penicillin (one million units every four hours). The wound on the left heel was debrided. Following one generalized seizure he was transferred to the Intensive Care Unit where he was treated with curare, and maintained on a respirator. Nine days after admission, it was decided to evaluate the condition of the patient after withdrawal of curare. Curare was stopped and atropine and neostigmine (Prostigmin) were given. The patient became bradycardic, developed atrioventricular block as well as asystole and died.

Autopsy findings

Autopsy was performed three hours after death. The right lower leg of this obese, well-developed black man had been surgically amputated, and the stump was well healed and clean. An ulcerative wound measuring 3 × 4 cm was present over the lateral left heel. The heart weighed 400 g; examination revealed severe arteriosclerotic changes of the coronary arteries, focal atheromatous segments narrowing the lumen. A proximal segment of the left circumflex coronary artery was completely occluded by two fresh brown thrombi. The posterior wall of the left ventricle displayed patchy areas of recent hemorrhagic infarction. The aorta, however, had only mild atherosclerotic changes. The left lung showed marked consolidation with microscopic findings of acute and subacute bronchopneumonia. Marked hepatosplenomegaly was present (liver, 23,500 g; spleen 650 g); microscopically moderate chronic congestion was noted. The gastrointestinal system was unremarkable. The kidneys showed the typical features of nodular intercapillary glomerular sclerosis characteristic of Kimmelstiel-Wilson disease. The pancreas was both grossly and microscopically unremarkable, as were the rest of the viscera.

Neuropathological findings. The brain weighed 1075 g, reflecting mild generalized cortical atrophy and focal cystic encephalomalacia (2 x 3 cm) in the right temporo-occipital region. The basal cerebral arteries showed mild atherosclerotic changes. The brain stem and the spinal cord were macroscopically

normal. Microscopically, the cortical neurons, including those of Ammon's horn, showed no ischemic or senile changes. The right temporo-occipital cortex showed focal cystic astrogliosis. The Betz cells in the motor strips appeared intact. Ascending tract degeneration was found throughout the spinal cord, especially the gracilis; and the posterior spinal roots showed diffuse interstitial fibrosis. The anterior spinal roots were spared and showed no sign of myelin or axonal damage. Most striking was the vacuolation concurrent with central chromatolysis, which was confined to the motoneurons of both the brain stem and spinal cord. The anterior horn motoneurons of the spinal cord, especially in the lumbar cord, were most severely involved; and almost all the anterior horn motoneurons showed honeycomb-like multiple vacuolations (*Fig. 1A and B*). The vacuoles measured 4–7 μ in diameter and often contained fine granules, which were readily seen in the Bodian stain (*Fig. 2A and B*). They occurred symmetrically throughout the spinal cord; the severity of involvement increased caudally. The symmetric distribution of lesions precluded the possibility of a secondary alteration from the right above-the-knee amputation. The neurons in Clark's columns, intermediolateral nuclei, and posterior horns were well preserved and free of vacuoles. The neurons in the brain stem motor nuclei were also involved to a lesser extent; motoneurons of XII, VII, and V displayed similar vacuolation. The neurons of sensory V, substantia nigra, reticular formation, locus ceruleus, and pontine gray were all intact. The vacuolation was often superimposed on centrally chromatolyzed large motoneurons. Chromatolysis (*Fig. 1A and B*), seldom without vacuoles, was also seen in the anterior horns on both sides. Small neurons in the anterior horns were generally spared (*Figs. 1A and 2A*). No astrocytic reaction was noted in the vicinity of affected neurons; neuronal loss was not apparent. No foci of perivascular demyelinative lesions or gliosis were noted.

Finely granular intravacuolar materials were visualized on silver impregnation (*Fig. 2A and B*). Transmission electron microscopy (TEM) revealed that the vacuoles contained finely granular or amorphous material and membrane-bound vesicles, 0.5 to 1.0 μ in diameter, containing parallel helical fibrillar material (*Fig. 3A and B*) and flocculant densities. These vesicles were considered to be altered mitochondria in the vicinity of vacuoles (*Fig. 3C*) where little autolytic change was seen in other subcellular organelles. The vacuoles themselves were seldom lined by a membrane. This impression was strengthened on scanning electron microscopy (SEM) of the specimens prepared according to the method described by Hamphery et al.[6] Cryofractured control motoneurons (*Fig. 4A*) revealed a finely granular fractured cytoplasmic surface without vacuoles; the nucleus with its chromatin and nucleolus was clearly identifiable. The vacuoles in the honeycombed neurons (*Fig. 4B*) were lined by a finely granular yet relatively smooth surface without a membrane. They infrequently displayed fine linear or granular contents over their surfaces (*Fig. 4B, arrows*), which corresponded in dimension to the vesicles seen on TEM.

Discussion

In his review of central nervous system (CNS) pathology in tetanus, Baker[4] cited several investigators around the turn of the century who had noted vacuolation in nerve cells. Müller and Jeschke[7] concluded that for induction of the vacuolation in the anterior horn cells

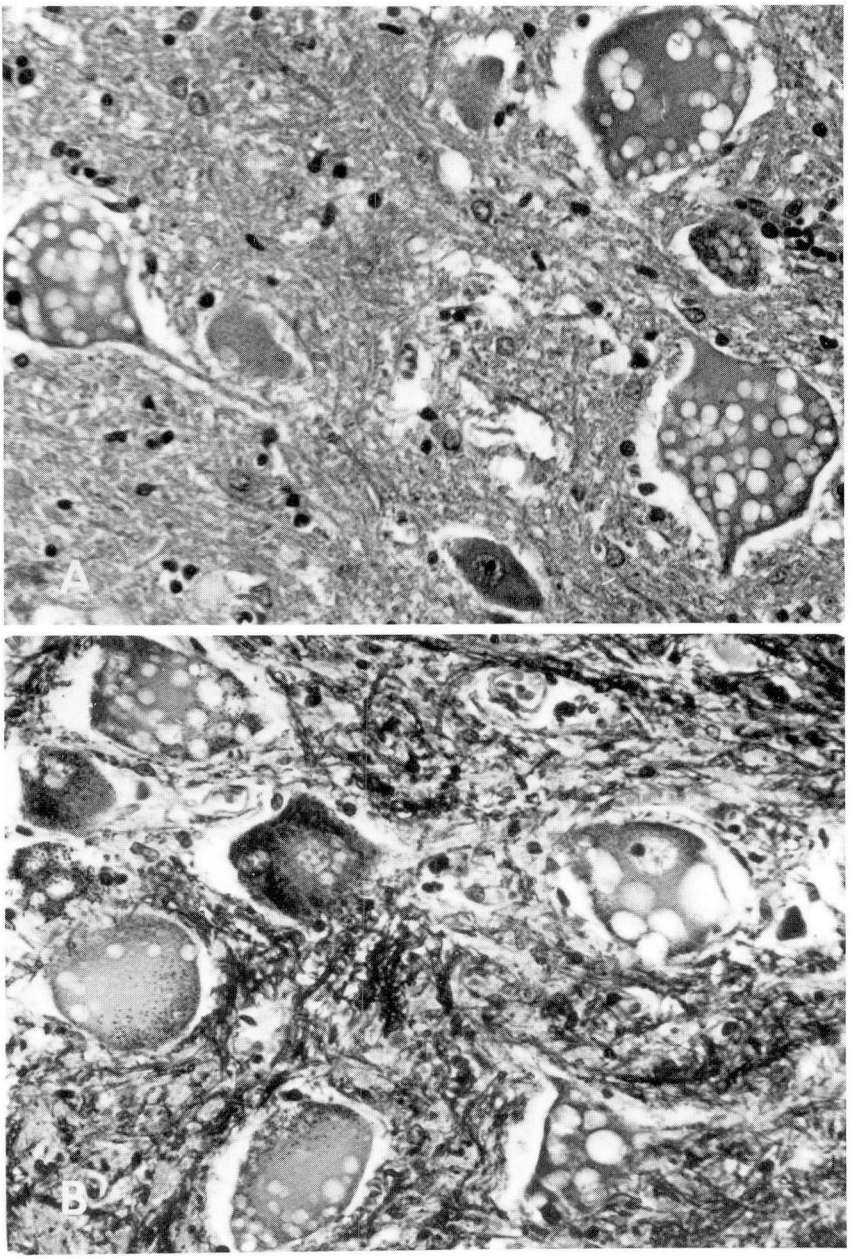

Fig. 1A. Large spinal motoneurons with multiple vacuoles in a honeycomb pattern; note relative sparing of small neurons; hematoxylin and eosin stain, × 360.

B. Motoneurons demonstrating both vacuolation and central chromatolysis; Kluver-Barrera stain, × 360.

(which was seen in 14 of 22 tetanus cases and in 2 of 50 cases without tetanus) two factors, hypoxia and tetanus toxin, should act simultaneously upon the motoneurons. Tetanus toxin may not be essential since transient, rhythmic, and synchronous myoclonic contraction of muscles secondary to an increased excit-

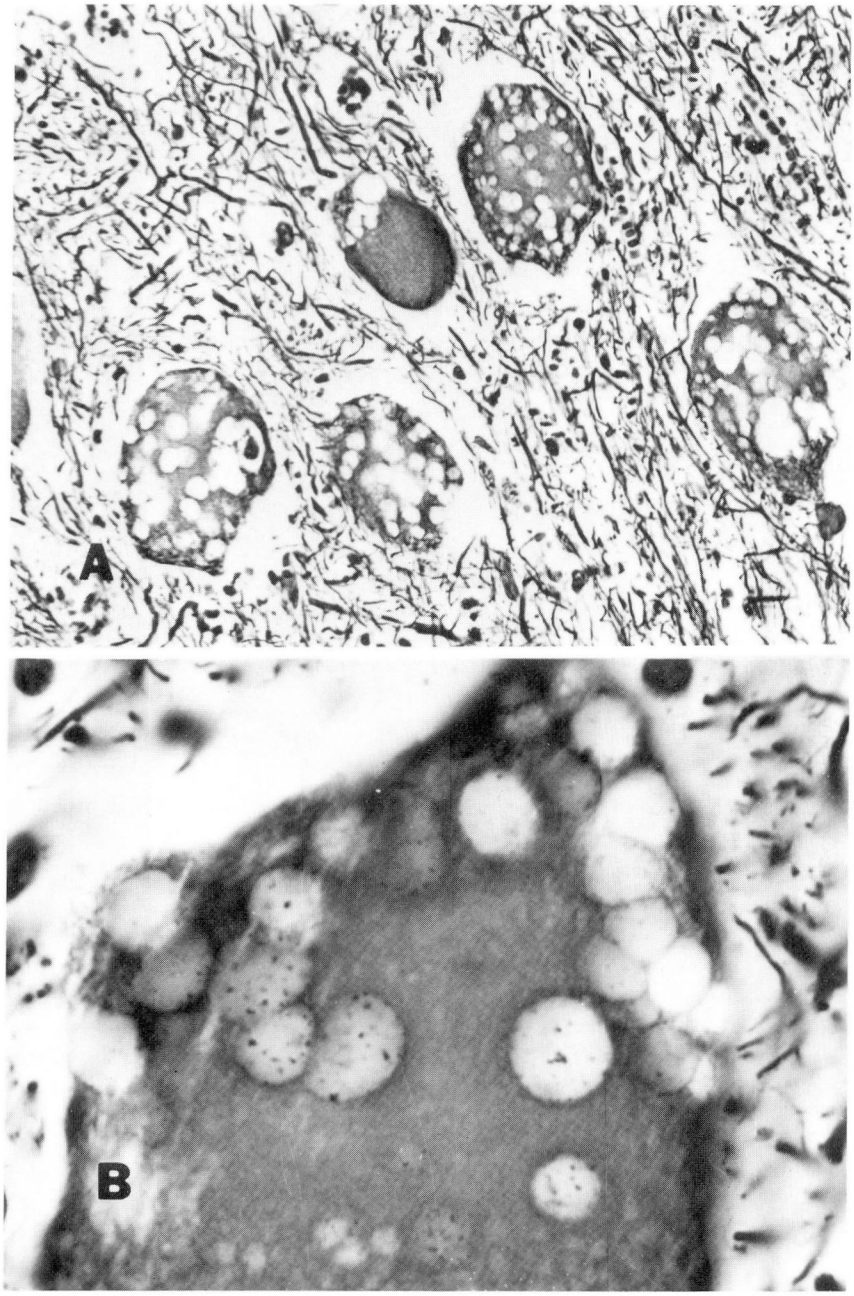

Fig. 2A. Honeycomb vacuolation accentuated by Bodian's silver impregnation; × 360.
B. Higher magnification of vacuoles showing fine granules within them; Bodian stain, × 1408.

ability of motoneurons is sufficient to induce vacuolation and chromatolysis.[8] The finding of chromatolysis in tetanus is more consistent than that of vacuolation and has been reported in both human and animal tetanus. Baker[4] described it in his 12 clinical cases and cited other investigators who have dem-

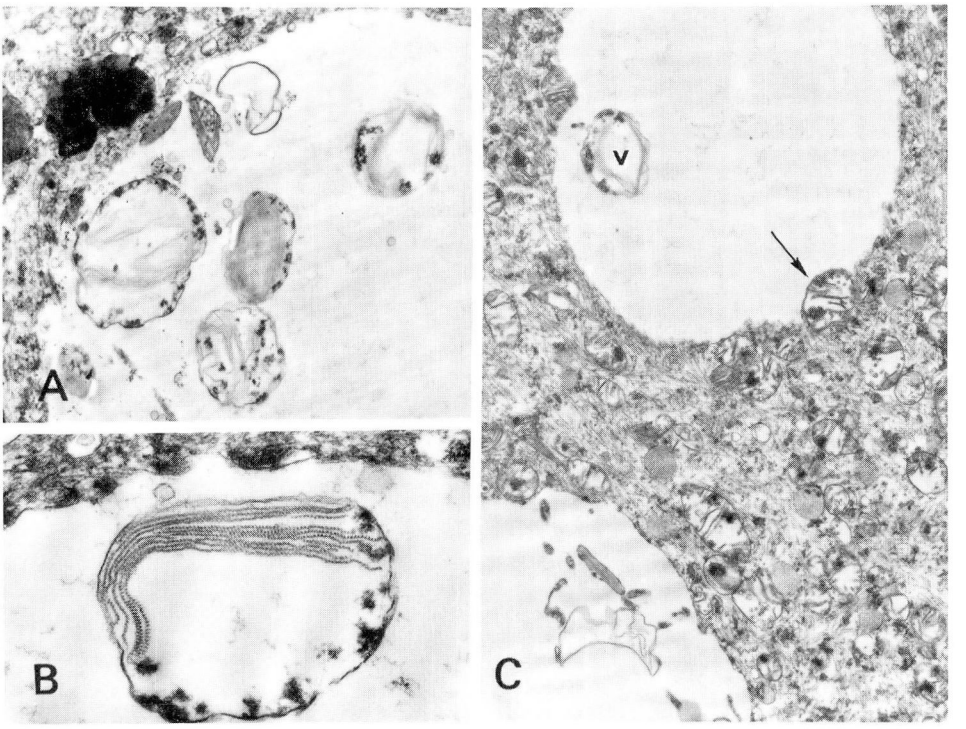

Fig. 3A. Round or spindle-shaped, membrane-bound bodies containing granules, cristae, and parallel helical fibrils within the vacuole, which is not membrane bound; × 35,000.

B. Detail of the membrane-bound round vesicular structure demonstrating parallel helical fibrillary material inside; × 52,500.

C. Lower magnification showing the suspected origin of the vesicle (V) from a mitochondrion with flocculant densities (arrow) protruding into the vacuole. Note relatively well-preserved subcellular organelles around the vacuole; × 18,000.

onstrated it. Tarlov[9] noted chromatolysis in local tetanus experimentally and hypothesized the causal relationship of this change to the continuous muscle rigidity clinically observed.

The vacuolation of nerve cells seen in the present case strikingly resembles that seen in 2 cases of infantile motoneuron disease.[10, 11] This disease was rapidly progressive with remarkable localization of vacuolation and central chromatolysis in the spinal and brain stem motoneurons, although present in Clark's column as well. As in the present case, vacuoles were often multiple within neurons and not associated with changes indicative of ischemic necrosis. The authors noted that the vacuolation occurred in cells in which Nissl granules were still present and thus probably either preceded or was independent of chromatolysis. Clinically, the patients demonstrated hypotonia and exhibited no rigidity or spasms, and both patients died of severe bronchopneumonia.

Our patient had periods of hypoxia-ischemia secondary to both bronchopneumonia and myocardial infarction shortly before death. However, the preferential involvement of large motoneurons in both the brain stem and spinal cord without morphologic changes of

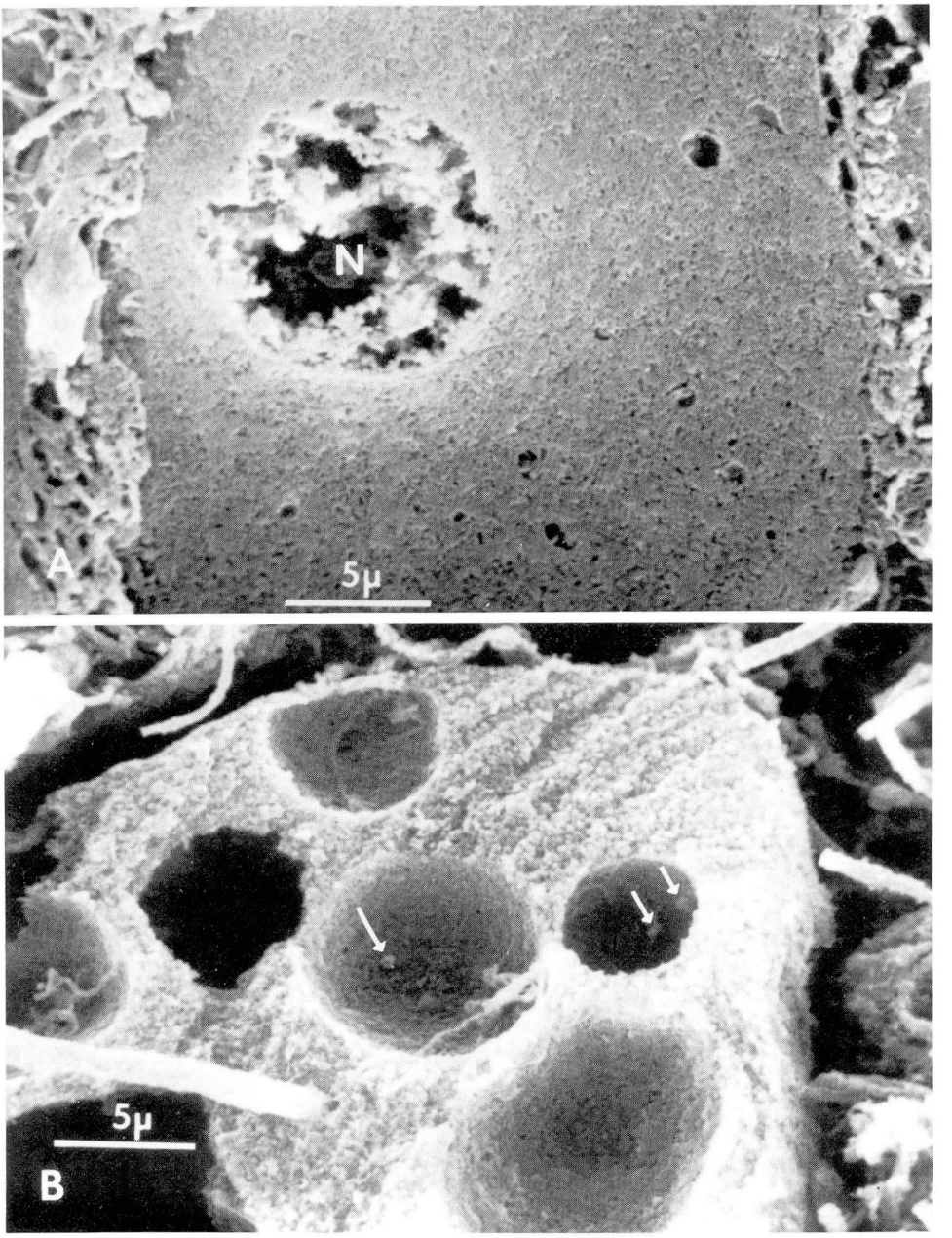

Fig. 4. Scanning electron micrograph of cryofractured spinal motoneuron. **A**. Nucleus and nucleolus (N) without large vacuolation in the cytoplasm of a control motoneuron.

B. Multiple vacuoles with granular or linear structures appear on the inner surfaces (arrows) of a honeycombed neuron.

ischemic necrosis is inexplicable on the basis of hypoxia-ischemia alone. Indeed, the selective involvement of large motoneurons argues against acute hypoxia-ischemia[12, 13] in which smaller neurons are more susceptible and large motoneurons are resistant. Furthermore, other hypoxia-ischemia changes such as homogenization, shrinkage, and incrustation do not coexist with vacuolation in the anterior horn or brain stem neurons of this patient; in fact, they appear free of ischemic changes. Besides, microvacuolation characteristic of earliest neuronal change in acute hypoxia-ischemia[13] differs markedly from the vacuoles in tetanus. The microvacuoles (diameter, 0.16–2.5 μ) occur in small cortical neurons,[14] whereas the vacuoles in tetanus are exclusively in the lower motoneurons and were never seen in Ammon's horn, Purkinje cells, inferior olivary, and dentate neurons.

Reported incidence of vacuolization is greater in more recently published tetanus cases. This may be because tetanus patients today live longer because of better supportive care,[7] as our patient who lived about ten days. Therefore, time would permit longer repetitive discharges from lower motoneurons. Fortunately, the phenomenon of vacuolization antedated tetanus toxoid so the toxoid would appear not to be a factor.

The electrophysiological evidence[15, 16] that tetanus toxin decreases inhibitory input to alpha motoneurons to cause increased firing and tetany is convincing. It appears as if the vacuolation and chromatolysis indicate severe damage by the toxin to motoneurons. However, these neurons must retain sufficient function to have the repetitive neuronal discharge or hyperexcitation necessary for tetanus. Indeed, base line physiological properties of motoneurons[17] or interneurons[18] in cats with local tetanus have been reported as normal.

The morphological changes in infantile motoneuron disease[13, 14] correspond closely to those in the tetanus cases described previously,[7] as well as to the present case, and raise the possibility that the mechanism induced by tetanus toxin could be related to motoneuron disease. Chromatolysis, basophilic clumping, and vacuolation have been observed in amyotrophic lateral sclerosis.[19, 20] The hypothesis that certain etiologic agents transported to the CNS via retrograde axonal transport, as is the case in tetanus poisoning,[1, 2] has been considered in motoneuron disease. The vacuoles formed in tetanus motoneurons fail to reveal any limiting membranes on both TEM and SEM, thus differing distinctively from those described in wobbler mouse,[21] Creutzfeldt-Jakob disease,[22] or distended mitochondria in anoxic-ischemia,[14] when they are membrane or multiple-membrane bound.

The intravacuolar membrane-bound vesicles appear to derive from degenerating mitochondria with flocculant densities (*Fig. 3C*). The diameters of 0.5–1.0 μ are compatible with those of mitochondria, and parallel helical fibrils have been described[23, 24] in mitochondria associated with nutritional deficiencies. The flocculant densities in the vesicles resemble those within the mitochondria (*Fig. 3C*) and those described by Zacks and Sheff[25] as tetanus-specific "intramitochondrial dense granules." In fact, similar intramitochondrial granules of high calcium content are typical of cells under hypoxia-ischemia of early autolysis. These mitochondrial changes may reflect a metabolic stress secondary to continuous hyperexcitability resulting from disinhibition of large motoneurons induced by tetanus toxin. The mechanism for cytoplasmic clearing and vacuole formation around the altered mitochondria remains obscure. The topistic restriction of the vacuolar changes

superimposing on chromatolysis in the lower motoneurons is puzzling and warrants further study in tetanus-intoxicated animal models.

Summary

Vacuolation and central chromatolysis overlapped and were found selectively in large motoneurons of the spinal cord and brain stem in a patient with clinically proved tetanus. From the histopathologic findings described in previously described tetanus patients, the inference was made that these concurrent conditions require the prolonged repetitive uninhibited discharges of the lower motoneurons that are induced by the effects of tetanus toxin. Ultrastructure of the vacuoles in tetanus motoneurons was compared with those of other conditions; they were not membrane-bound and contained vesicles enclosing granules and parallel helical fibrils, probably products of degenerated mitochondria. Hence the vacuoles were distinctively different from those microvacuoles formed by membrane-bound distended mitochondria in acute hypoxia.

Acknowledgment

This research was carried out during Dr. Payne's postdoctoral fellowship from the Muscular Dystrophy Association.

References

1. Bizzini B. Tetanus toxin. Microbiol Rev 1979; **43:** 224–240.
2. Habermann E. Tetanus. In: Vinken PJ, Bruyn GW, eds. Handbook of Clinical Neurology. Amsterdam: North Holland 1978: 491–547.
3. Foster JA, Matzke HA. The neurocytology of experimental ascending tetanus. World Neurol 1961; **2:** 22–35.
4. Baker AB. Medullary involvement in tetanus. Am J Pathol 1943; **19:** 709–717.
5. Tarlov IM, Ling H, Yamada H. Neuronal pathology in experimental local tetanus; clinical implications. Neurology 1973; **23:** 580–591.
6. Hamphery WJ, Spurlock BO, Johnson JS. Critical point drying of ethanol-infiltrated cryofractured biological specimens for scanning electron microscopy. In: Proc 7th Annual SEM Symposium, Part 1. Chicago: IIT Research Inst 1974: 275–282.
7. Müller HA, Jeschke R. Cytoplasmatische Vacuolen in den Vorderhornganglienzellen des Menschen beim Tetanus. Virchows Arch 1971; **353:** 87–98.
8. Shavapour E, Teasdall RD. Spinal myoclonus with vacuolar degeneration of anterior horn cells. Arch Neurol 1980; **37:** 451–453.
9. Tarlov IM. Rigidity and primary motoneuron damage in tetanus. Exp Neurol 1974; **44:** 246–254.
10. Kohn R. Clinical and pathological findings in an unusual infantile motor neuron disease. J Neurol Neurosurg Psychiat 1971; **34:** 427–431.
11. Reif-Kohn R, Mundel G. Second case of an infantile motor neuron disease. Confin Neurol 1974; **36:** 23–32.
12. Lindenberg R. Morphotrophic and morphostatic necrobiosis; investigations on nerve cells of the brain. Am J Pathol 1956; **32:** 1147–1168.
13. Brierley J. Cerebral hypoxia. In: Blackwood W, Corsellis A, eds. Greenfield's Neuropathology. London: Edward Arnold, 1976: 43–85.
14. Brown AW, Brierley JB. Anoxic-ischaemic cell change in rat brain; light microscopic and fine-structural observations. J Neurol Sci 1972; **16:** 59–84.
15. Semba T, Kano M. Glycine in the spinal cord of cats with local tetanus rigidity. Science 1969; **164:** 571–572.
16. Curtis DR. The pharmacology of spinal postsynaptic inhibition. Progr Brain Res 1969; **31:** 171–189.
17. Wiegand H, Wellhöner HH. Electrical excitability of motoneurons in early local tetanus. Naunyn Schmiedebergs Arch Pharmacol 1979; **308:** 71–76.
18. Benecke R, Takano K, Schmidt J, Henatsch HD. Tetanus toxin induced actions on spinal Renshaw cells and Ia-inhibitory interneurons during development of local tetanus in the cat. Exp Brain Res 1977; **27:** 271–286.
19. Chou SM. Pathognomy of intraneuronal inclusions in ALS. In: Tsubaki T, Toyokura Y, eds. Amyotrophic Lateral Sclerosis. Tokyo: Univ Tokyo Press, 1979: 135–176.
20. Hirano A, Iwata M. Pathology of motor neurons with special reference to amyotrophic lateral sclerosis and related disease. In: Tsu-

baki T, Toyokura Y, eds. Amyotrophic Lateral Sclerosis. Tokyo: Univ Tokyo Press, 1979: 107–132.
21. Andrews JM. The fine structure of the cervical spinal cord, ventral root and brachial nerves in the wobbler (wr) mouse. J Neuropath Exp Neurol 1975; **34:** 12–27.
22. Chou SM, Payne WN, Gibbs CJ Jr, Gajdusek DC. Transmission and scanning electron microscopy on spongiform change in Creutzfeldt-Jakob disease. Brain 1980; **103:** 885–904.
23. Porta EA, Hartroft WS, de la Iglesia FA. Hepatic changes associated with chronic alcoholism in rats. Lab Invest 1965; **14:** 1437–1455.
24. Svoboda D, Higginson J. Ultrastructural changes produced by protein and related deficiencies in the rat liver. Am J Pathol 1964; **45:** 353–379.
25. Zacks SF, Sheff MI. Studies on tetanus toxin. 1. Formation of intramitochondrial dense granules in mice acutely poisoned with tetanus toxin. J Neuropath Exp Neurol 1964; **23:** 306–323.