Influence of Neostigmine Treatment on Embryonic Development of Acetylcholine Receptors and Neuromuscular Junctions

G. S. SOHAL and W. R. BOYDSTON
Department of Anatomy, Medical College of Georgia, Augusta, Georgia 30912

ABSTRACT The postulated role of the acetylcholine receptor in the formation of neuromuscular synapses during the course of embryonic development was investigated in the superior oblique muscle of white Peking duck embryos. The possibility that the number of receptors could be experimentally lowered by chronic injections of the anticholinesterase agent, neostigmine methylsulfate, was determined using $^{125}$I-a-bungarotoxin. The total number of acetylcholine receptors on incubation day 12, 2 d subsequent to the onset of treatment, was reduced 45% as compared to saline-treated controls. A similar reduction in total receptor content (49%) was also observed on day 19. Radioautographic preparations showed that clusters of acetylcholine receptors were rare and that the grain density of extrajunctional receptors was also reduced. Hence, chronic treatment with neostigmine during development was observed to exert an effect on both the number and distribution of receptors in the developing superior oblique muscle. These changes occurred in the absence of any apparent effect on muscle differentiation in general. Myoblasts and myotubes were present on day 14 and further differentiated into myofibers by day 18 in both neostigmine and saline-treated muscles. The cytology of the developing muscle cells also appeared normal. This is in contradistinction to the striking morphological changes that take place in adult mammalian and avian muscle after anticholinesterase treatment. More significantly, the decreased total receptor content and sparsity of clusters had no apparent effect on the formation of developing neuromuscular junctions at the electron microscopic level. The frequency of neuromuscular junctions in neostigmine-treated muscles was similar to that of the controls. It is concluded that acetylcholine receptor clusters are not required for the events leading to the morphological formation of neuromuscular junctions during in vivo development.

Although initially developing independently, a skeletal muscle and its motor nerve become structurally and functionally dependent on each other during subsequent embryonic development through a complex series of interactions eventually leading to the formation of neuromuscular junctions (NMs). The mechanisms by which NMs are established, either during the course of embryonic development or after reinnervation of adult denervated muscle, are little understood. One of the extensively investigated aspects of the NMJ is the presence of acetylcholine receptor (AchR) molecules in the plasma membrane of the muscle fiber. In relatively immature muscle cells the AchRs are distributed uniformly over the entire sarcolemma (7, 17). An integral part of the maturation process of the developing muscle is the change in distribution and number of AchRs, and in many properties of the receptor such as metabolic turnover times, channel open times, and immunological properties (9, 10, 19, 22, 48, 51, 67). At about the time of the initial innervation, aggregates of AchRs (clusters or hot spots) appear in addition to the relatively low density receptors (7, 8). Although temporally coincidental, the initial appearance of clusters of receptors is not dependent on innervation because the receptor clusters appear on myotubes grown in culture in the absence of neural tissue and in aneural muscle (3, 4, 8, 21, 55, 63). In vitro studies suggest that the growing nerve fibers induce new receptor clusters at the point of nerve-muscle contact (3, 24). Gradually, during the course of embryonic development, the low density receptors disappear and almost all AchRs in the adult muscle fiber are localized to the region...
of the NMJ (2, 19, 29, 42). One of the intriguing questions pertaining to synaptogenesis is what role, if any, do the AchR clusters play in formation of the NMJ?

In this paper we report the effects of neostigmine methylsulfate on the embryonic development of AchRs, formation and maintenance of NMJs, and muscle differentiation. Neostigmine methylsulfate and other anticholinesterase drugs have been shown to produce postsynaptic morphological, biochemical, and electrophysiological alterations in adult mammalian skeletal muscle after acute and long-term treatment (1, 18, 20, 23, 30, 34, 37, 38, 49, 52, 64, 66). Initially, the myopathic changes are localized to the junctional region and include disruption of the myofibrillar apparatus, swelling of subjunctional membrane-bound organelles, and a simplification of postsynaptic folds (18, 30, 34, 52). With continued treatment, the degenerative changes spread to regions adjacent to the motor endplate. Furthermore, chronic treatment of adult mammalian muscle with neostigmine reduces the total number of acetylcholine receptors in the range of 42-45% in the endplate region (12, 44). Whether or not neostigmine also decreases the total number of AchRs in the embryonic skeletal muscle and alters muscle morphology during in vivo development is unknown.

MATERIALS AND METHODS

Animals and Drug Regimen

White Peking duck embryos were treated with 1-5 μg neostigmine methylsulfate (prostigmin, Roche Laboratories, Hoffmann-LaRoche, Inc., Nutley, NJ) per gram body weight. The drug solution was placed on the vascularized choroido-lantoic membrane through an opening in the shell beginning on day 10 and continuing daily until sacrifice. Previous studies have shown that the out-growing trochlear nerve fibers reach their target, the superior oblique muscle, on day 10; neuromuscular transmission begins on day 11, and primitive neuromuscular junctions first become visible on day 13 (56, 58, 59, 62). Control embryos received equal amounts of a sterile, physiological saline solution over the same time period.

Acetylcholine Receptor Binding and Autoradiography

Acetylcholine receptor quantification was performed by conjugating Na¹²⁵I (Amersham Corp., Arlington Heights, IL) with α-bungarotoxin (α-BTX, Miami Serpentarium, Miami, FL) using the chloramine-T method (5, 28). Upon sacrifice muscles were quickly dissected and each muscle was incubated at 4°C for 4 h in 1 ml Locke's solution containing 0.3 μg ¹²⁵I-α-BTX (sp act 57 Ci/mM). Subsequently, the muscles were washed overnight in Locke's solution at 4°C to remove unbound toxin. The amount of bound ¹²⁵I-α-BTX (hence receptor content) was determined with a gamma counter. The nonspecific binding in our preparations, as determined by preincubation of muscles with cold toxin, was 7%. Total muscle protein was determined from muscle homogenates by the method of Lowry et al. (40) using bovine serum albumin as the standard.

For autoradiography the superior oblique muscles were labeled and washed as described above with the one exception that an iodinated toxin of higher specific activity was used (200 Ci/mM, Amersham Corp.). Subsequent to washing, the muscles were fixed for 4 h in 4% paraformaldehyde-glutaraldehyde solution. They were then washed in Locke's solution for 6 h at 4°C, frozen in liquid nitrogen, and longitudinally sectioned on a cryostat at 20 μm. Sections were coated with NTB-2 liquid emulsion (Kodak) diluted 1:1 with water. After a 6-d exposure at 4°C, the slides were hydrated, developed for 3 min at 18-20°C (2 parts H₂O to 1 part D19), and fixed. The slides were then lightly stained with hematoxylin and eosin and cover-slipped. Muscles on embryonic days 11, 16, 19, and 23 were processed for autoradiography. Quantitative autoradiographic studies were performed on embryonic day 23. AchR clusters were counted with the aid of a camera lucida drawing tube attached to a light microscope. Silver grain counts of extrajunctional AchRs were made from photographs at a final magnification of 850 times. The areas were measured with a Zeiss MOP-1.

Light and Electron Microscopy

For electron microscopy, muscles were fixed overnight in 4% paraformalde-
normal muscles were preincubated with neostigmine for 1 h. No significant differences in the total number of receptors between the treated and nontreated muscles were observed suggesting that neostigmine does not inhibit α-BTX binding to the receptor. Injections of neostigmine during the course of embryonic development therefore appeared to have an identical effect on the total number of acetylcholine receptors as reported in adult muscle (12, 44).

In contrast to adult innervated muscle, embryonic muscle is characterized by populations of both junctional and extrajunctional receptors. Thus, the question arises as to how the neostigmine-induced reduction in receptor number is affecting receptor distribution. Fig. 2a shows the typical pattern of receptor distribution on day 23 in saline-treated controls. In

![Figure 2](https://example.com/figure2.jpg)

**Figure 2** Autoradiographic demonstration of acetylcholine receptor distribution in superior oblique muscle on embryonic day 23. *a*, control; *b*, neostigmine-treated. Note the sparsity of both clusters of receptors as well as extrajunctional receptors in *b*. X 525.
addition to the extrajunctional receptors, obvious receptor clusters were seen. However, such clusters were sparse in neostigmine-treated muscles (Fig. 2b). Quantitative study of receptor clusters indicated that ~85% of the clusters were absent in the neostigmine-treated embryos (Table I). When present, the area of the remaining clusters was considerably smaller (Table II). Counts of silver grains indicated that the extrajunctional receptors were also fewer in number in neostigmine-treated embryos (Table II). Thus, reduction in total number of receptors after neostigmine treatment is due to loss of both clusters of receptors and extrajunctional receptors.

Effects of Neostigmine on Neuromuscular Junction Formation and Muscle Differentiation

To verify the anticholinesterase nature of neostigmine in a developing muscle system, an acetylcholinesterase stain was performed. By staining cholinesterase deposits at the junctional regions, this procedure allows for the demonstration of motor endplates at the light microscopic level. In Fig. 3a, distinct acetylcholinesterase deposits are seen in the control muscle on day 23. This staining was absent when tissues were preincubated with neostigmine for 30 min. Normally, the first appearance of endplates detectable in the superior oblique muscle with this stain is on incubation day 18. On the other hand, an absence of distinct acetylcholinesterase staining of motor endplates was noticed in neostigmine-treated embryos despite repeated tissue washing of up to 2 h (Fig. 3b (possible explanations for lack of cholinesterase staining in neostigmine-treated muscles and difficulties with this experiment are considered in the last paragraph of discussion). Upon electron microscopic examination of treated muscles on days 14, 16, 19, and 23, neuromuscular junctions appeared normal morphologically and their temporal appearance coincided with those observed in saline-treated control muscle (Fig. 4a). Hence, no differences in the time of appearance or structure of developing neuromuscular junctions occurred. In view of the loss of a vast majority of clusters it was of considerable interest to determine whether or not the number of neuromuscular junctions in neostigmine-treated embryos was comparable to that of the control. An ideal approach would be to count the number of motor endplates from muscles stained with a combined acetylcholinesterase and silver impregnation method at the light microscope level. Unfortunately, lack of acetylcholinesterase staining in neostigmine-treated muscles precluded this type of analysis. However, some indication of the frequency of neuromuscular junctions was obtained using quantitative electron microscopy. Table I indicates that the frequency of findings neuromuscular junctions in the treated embryos was similar to that of the controls.

A cursory look at the neostigmine-treated embryos at the time of injections gave the impression of normal quantitative motility. However, totally different results were obtained when the number of limb movements were quantitated in a systematic fashion. Table III indicates that embryonic movements were significantly reduced in the treated embryos. Furthermore, this reduced level of activity remained virtually unchanged in the neostigmine-treated embryos whereas a progressive increase in the number of limb movements occurred in the control embryos.

As striking changes in the organization of contractile elements and degeneration of postsynaptic folds occur in adult muscle after long-term anticholinesterase treatment, the effect of neostigmine on muscle differentiation was examined. The normal stages in the development of the superior oblique have been well documented and were not seen to vary in neostigmine-treated embryos (59). Myoblasts and myotubes were present on day 14 and further differentiated into myofibers by day 18. The cytology of the developing muscle fibers was also normal in the sense that the appropriate complement of organelles was seen and myofilaments became well organized and assembled into myofibrils (Fig. 4b). Numerous intramuscular nerve bundles of normal ultrastructure were encountered.

Our failure to observe massive morphological alterations in neostigmine-treated embryonic muscles suggested that either the embryonic muscle is not as susceptible to anticholinesterase myopathy as the adult muscle or that for some reason the anticholinesterase myopathy does not occur in avian muscles. Since all of the reported anticholinesterase myopathy studies were done on mammals we examined the effects of neostigmine treatment on the adult duck muscle. Adult ducks were given intraperitoneal injections (0.05 mg/kg body weight in 0.1 ml solution of neostigmine methylsulfate) twice daily for 7 d. Shivering and salivation was observed shortly after the injections. Ducks injected with 0.25 mg/kg body weight of neostigmine of 0.5 ml solution died within 10 min of injection. After sacrifice, superior oblique muscles were examined with electron microscopy. Myopathic changes similar to those reported for the adult mammalian muscle were observed and a limited evidence of this is provided in Fig. 5.
DISCUSSION

The results of the present study indicate that continued treatment of the embryo with neostigmine before, during, and after the period of synaptogenesis in the superior oblique muscle significantly reduces the total number of AchRs. Despite such a reduction in AchRs and the absence of clusters of receptors, neuromuscular junctions of normal ultrastructure were observed. Further, the progressive differentiation of the myogenic cells to the myofiber stage was unaffected as a result of neostigmine treatment. These findings suggest that the total receptor content and the ability of receptors to cluster may not be essential elements either for the formation and maintenance of the morphological aspects of the neuromuscular junctions or for the growth and maturation of the muscle. Whether the neuromuscular junctions were functionally normal in the treated embryos could not be ascertained with the present approach. This could only be determined by electrophysiological recordings. Reduced levels of embryonic motility can be interpreted to mean fewer physiologically normal junctions.
However, the drastic reduction in the total number of receptors and the fact that most junctions are without AchR clusters could also account for reduction in activity.

Our observations are consistent with findings in other systems regarding the role of AchRs in synapse formation. A study by Anderson et al. (3, 4) has suggested that the clusters of receptors may be formed along the course of neurite-muscle contacts in cultured Xenopus myotubes. A detailed mapping study of AchR clusters on chick myotubes grown in culture was recently carried out by Frank & Fischbach (24). By mapping AchR clusters before and after synapse formation, they observed that the ingrowing nerve fibers induced new clusters of receptors rather than seeking out the preexisting ones. The uninnervated AchR clusters gradually disappeared.
suggesting that the initial AchR clusters may not represent preferred sites for synapse formation. It is now clear that the growing neurites induce clusters of receptors at the region of transmitter release. The mechanism by which neurites induce this clustering is as yet unknown. Several reports indicate that a factor of neural origin may be responsible for clustering of receptors (13, 15, 33, 47). Extracts of spinal cord or brain have been shown to contain a factor that increases the total number of AchRs and the number of receptor clusters on muscle cells grown in culture (33, 47). Media conditioned by neuroblastoma...
× glioma hybrid cells can also increase the total number of AchR clusters on myotubes in culture (13). The molecular mechanism underlying the action of these factors is completely unknown.

When embryonic muscle cells are cultured with neurons in the presence of curare, synapses are formed (14, 45, 50). Similarly, when neuromuscular transmission is blocked with pre- or postsynaptic blocking agents (such as botulinum toxin, hemicholinium, and bungarotoxin), neuromuscular junctions of normal ultrastructure develop in vitro (25, 26, 56, 59, 60). In vitro studies by Steinbach et al. (61) on the clonal lines of nerve hemicholinum,.

unknown.

Similarly, when neuromuscular transmission is blocked with pre-or postsynaptic blocking agents (such as curare, synapses are formed (14, 45, 50). When embryonic muscle cells are cultured with neurons in the presence of curare, synapses are formed (14, 45, 50). Similarly, when neuromuscular transmission is blocked with pre-or postsynaptic blocking agents (such as curare, synapses are formed (14, 45, 50).

AchR clusters on myotubes in culture (13). The molecular mechanism underlying the action of these factors is completely unknown.

We are grateful to Beth McBride, Greg Oplak, and Tenna Knox for their excellent technical assistance and to Rosemary Widener for her careful typing of the manuscript.

This work was supported by grants from National Institutes of Health and Muscular Dystrophy Association.

Received for publication 17 August 1981, and in revised form 10 May 1982.

REFERENCES

1. Ariens, A. TH., E. Meeter, O. L. Weelhuis, and R. M. J. van Benthem. 1969. Reversible necrosis at the end-plate region in striped muscles of the rat poisoned with cholinesterase inhibitors. *Experientia* (Basel). 25:57-59.

2. Axelsson, J., and S. Thesleff. 1959. A study of supersensitivity in desensitized mammalian skeletal muscle. *J. Physiol.* (Lond.). 147:178-193.

3. Anderson, M. J., and M. W. Cohen. 1977. Nerve-induced and spontaneous redistribution of acetylcholine receptors on cultured muscle cells. *J. Physiol.* (Lond.). 268:737-773.

4. Anderson, M. J., M. W. Cohen, and E. Zorychta. 1977. Effects of innervation on the distribution of acetylcholine receptors on cultured muscle cells. *J. Physiol.* (Lond.). 268:731-756.

5. Berg, D. K., B. B. Kelly, P. B. Sargent, P. Williamson, and Z. W. Hall. 1972. Binding of α-bungarotoxin to acetylcholine receptors in mammalian muscle. *Proc. Natl Acad. Sci. U. S. A.* 69:147-151.

6. Betz, W., and B. Saikman. 1973. Effects of proteolytic enzymes on function and structure of frog neuromuscular junctions. *J. Physiol.* (Lond.). 230E:673-688.

7. Bean, S., and J. H. Steinbach. 1977. The distribution of α-bungarotoxin binding sites on mammalian skeletal muscle developing in vivo. *J. Physiol.* (Lond.). 267:95-215.

8. Brandauer, A. W., and A. J. Harris. 1979. Neuronal influence on acetylcholine receptor clusters during embryonic development of skeletal muscles. *Nature* (Lond.). 279:549-551.

9. Burden, S. 1977. Development of the neuromuscular junction in the chick embryo: the number, distribution, and stability of acetylcholine receptors. *Dev. Biol.* 57:317-329.

10. Burden, S. 1977. Acetylcholine receptors at the neuromuscular junction: developmental change in receptor turnover. *Dev. Biol.* 61:79-95

11. Burden, S. J., P. B. Sargent, and U. J. McMahan. 1979. Acetylcholine receptors in...
regenerating muscle accumulate at original synaptic sites in the absence of the nerve. J. Cell Biol. 82:125-127.
12. Chang, C. C., T. F. Chen, and S. T. Chuang. 1973. Influence of chronic neostigmine treatment on the number of acetylcholine receptors and the release of acetylcholine from cat diaphragm. J. Pharmacol. Exp. Ther. 194:557-564.
13. Christian, N. C., M. P. Daniels, H. Sugiyama, Z. Vogel, L. Jacques, and P. G. Nelson. 1978. A factor from neurons that increases the number of acetylcholine receptor aggregates in cultured muscle cells. Proc. Natl. Acad. Sci. U. S. A. 75:402-406.
14. Cohen, M. W. 1972. The development of neuromuscular connections in the presence of d-tubocurarine. Brain Res. 41:487-463.
15. Cohen, S. A., and G. D. Fischbach. 1977. Relative peaks of ACh sensitivity at identified nerve-muscle synapses in spinal cord muscle cell cultures. Dev. Biol. 59:34-38.
16. Creazzo, T. L., and G. S. Sohal. 1981. Beta-bungarotoxin lacking phospholipase activity is not toxic to developing motor neurons and skeletal muscle. Dev. Neurosci. 4:229-236.
17. Diamond, J., and R. Miledi. 1962. A study of foetal and new-born rat muscle fibers. J. Physiol. (Lond.) 162:391-400.
18. Engel, A. H., E. H. Lambert, and T. Santa. 1973. Study of long-term anticholinesterase therapy. Neurology. 23:1273-1281.
19. Fambrough, D. M. 1979. Control of acetylcholine receptors in skeletal muscle. Physiol. Rev. 59:165-227.
20. Fischbach, G. D., and S. A. Cohen. 1973. The distribution of acetylcholine sensitivity over uninnervated and innervated muscle fibers grown in cell culture. Dev. Biol. 31:147-162.
21. Fischbach, G. D., and S. M. Schuetze. 1980. A postnatal decrease in acetylcholine channel open time at endplates. J. Physiol. (Lond.) 303:125-138.
22. Fischer, G. 1976. Die Acetylcholinesterase an der motorischen Endplattene des Ratzenwurmkopf- fells nach Injektion mit Parathion und Soman bei Applikation von Oximet. Experientia (Basel) 32:402-403.
23. Frank, E., and G. D. Fischbach. 1979. Early events in neuromuscular junction formation and development of the postjunctional membrane in cultured muscle cells. J. Gen. Physiol. 62:286-382.
24. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1962. The proportion of 1H-labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114-116.
25. Hamburger, H., and F. F. Halford. 1972. Acetylcholine receptors, distribution, and extrajunctional density in rat diaphragm after denervation correlated with acetyleholine sensitivity. J. Gen. Physiol. 60:340-356.
26. Hansen, J. K., and D. C. van Essen. 1975. Reinnervation of rat skeletal muscle. Cell Tissue Res. 163:215-223.
27. Kawabuchi, M., M. Osamu, S. Watanabe, A. Igata, and T. Kanazawa. 1976. Myopathic changes at the end-plate region induced by neostigmine methylsulphate. Europ. J. Biochem. 62:623-625.
28. Keshavarz, Y., and K. Yab. 1981. Synaptic contacts between embryonic Xenopus neurons and myotubes formed from a rat skeletal muscle cell line. Dev. Biol. 86:12-16.
29. Koegel, J., and M. Vigga. 1978. Neural induction of the 185 acetylcholinesterase in muscle cell cultures. Nature (Lond.) 271:75-77.
30. Lakowski, M. B., W. H. Olson, and D. D. Dethorn. 1977. Initial ultrastructural abnormalities at the motor endplate produced by a cholinesterase inhibitor. Exp. Neurol. 57:13-23.
31. Leonard, J. P., and M. D. Hal. 1979. Agonist-induced myopathy at the neuromuscular junction is mediated by calcium. J. Cell Biol. 82:811-819.
32. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.
33. Marshall, L. M., J. R. Sanes, and U. J. McMahan. 1977. Reinnervation of original synaptic sites on muscle fiber basement membrane after disruption of the muscle cells. Proc. Natl. Acad. Sci. U. S. A. 74:7037-7041.
34. Miledi, R. 1960. Functional and extrafunctional acetylcholine receptors in skeletal muscle fibers. J. Physiol. (Lond.) 151:24-30.
35. Moody-Corbett, F., and M. W. Cohen. 1981. Localization of cholinesterase at sites of high acetylcholine receptor density on embryonic amphibian muscle cells cultured without nerves. J. Neurol. 259:605-610.
36. Noble, M. D., J. H. Peacock, and W. W. Hofmann. 1979. Predosinone-Neostigmine interactions at cholinergic junctions. Muscle Nerve. 2:155-157.
37. Obera, K. 1977. Development of neural musculature in culture with a variety of neurons and in the presence of cholinergic substances and TTX. Brain Res. 119:141-153.
38. Pittman, R., and R. W. Oppenheim. 1979. Cell death of motoneurons in the chick embryo spinal cord. J. Neurocytol. 18:425-446.
39. Podlaski, R. T., R. D. Axelrod, P. Ravdin, I. Greenberg, M. M. Johnson, and M. M. Salpeter. 1979. Nerve- induced increase and redistribution of acetylcholine receptors on cholinergic nerve cells. Proc. Natl. Acad. Sci. U. S. A. 75:2035-2039.
40. Prinzen, G. C., and Z. W. Hall. 1980. The developmental change in immunological properties of the acetylcholine receptor in rat muscle. Dev. Biol. 81:304-311.
41. Roberts, D. V., and S. Thesleff. 1969. Acetylcholine release from motor-nerve endings in rats treated with neostigmine. Eur. J. Pharmacol. 2:281-285.
42. Ruben, L. L., S. M. Schuetze, C. L. Weil, and G. D. Fischbach. 1980. Regulation of acetylcholinesterase appearance at neuromuscular junctions in vivo. Nature (Lond.) 281:264-267.
43. Sakmann, B., and H. R. Benny. 1978. Changes in synaptic channel during neuromuscular development. Nature (Lond.) 278:401-402.
44. Sanes, J. R., M. M. Haas, P. G. M. M. Haas, and H. Frett. 1979. Endplates after esterase inactivation in vivo: correlation between esterase concentration, functional response, and fine structure. J. Neurocytol. 8:95-115.
45. Sand, J. R., and Z. W. Hall. 1980. Antibodies that bind specifically to synaptic sites on muscle fiber basal lamina. J. Cell Biol. 83:370-372.
46. Sanes, J. R., L. M. Marshall, and U. J. McMahan. 1978. Reinnervation of muscle fiber basal lamina after removal of myotubes. Differentiation of regenerating axons at original synaptic sites. J. Cell Biol. 87:178-198.
47. Sohal, G. S. 1981. Neural influence on embryonic skeletal muscle. Anat. Rec. 199:242A (Abstr.).
48. Sohal, G. S. 1981. Ultrastructural observations on the establishment of neuromuscular junctions following prevention of embryonic death of motoneurons. Exp. Neurol. 71:625-632.
49. Soja, G. S., T. L. Creazzo, and T. G. Olsbak. 1979. Effects of chronic paralysis with a-bungarotoxin on development of innervation. Exp. Neurol. 66:619-626.
50. Soja, G. S., and R. K. Holt. 1980. Role of innervation on the embryonic development of skeletal muscle. Cell Tissue Res. 205:383-393.
51. Stroka, T., and G. Vroba. 1978. The role of muscle activity in the differentiation of neuromuscular junctions in the slow and fast twitch muscles. J. Neurocytol. 7:529-540.
52. Stroka, T., and G. Vroba. 1978. The role of muscle activity in the differentiation of neuromuscular junctions in the slow and fast twitch muscles. J. Neurocytol. 7:529-540.
53. Stroka, T., and G. Vroba. 1978. The role of muscle activity in the differentiation of neuromuscular junctions in the slow and fast twitch muscles. J. Neurocytol. 7:529-540.
54. Stroka, T., and G. Vroba. 1978. The role of muscle activity in the differentiation of neuromuscular junctions in the slow and fast twitch muscles. J. Neurocytol. 7:529-540.