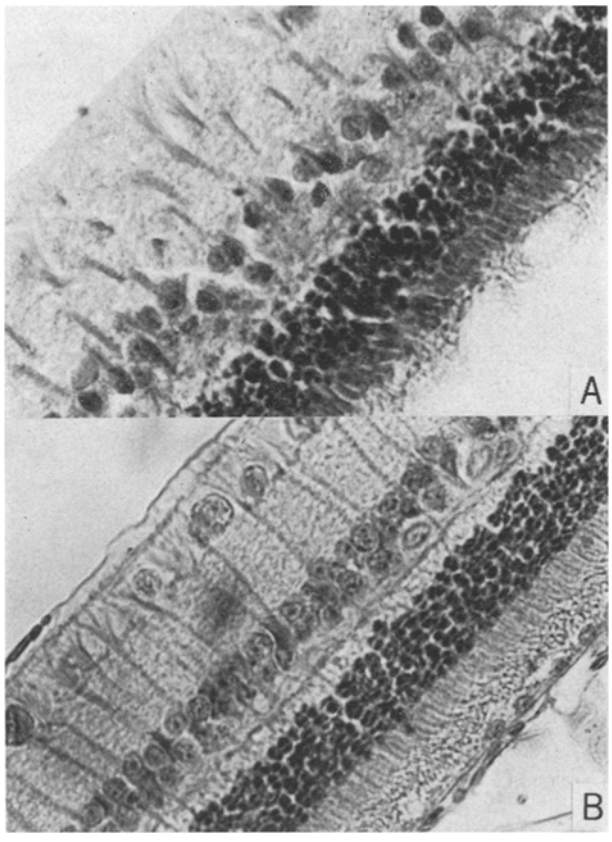

Fig. 2. Light microscopic observation on the retina of rabbit exposed to high concentration of oxygen for 40 h (A) and that of the control (B).

As shown in Figure 1, the exposure to oxygen for 12 h resulted in the increase in lipoperoxide in the retina as compared with the control ($p < 0.02$). Upon continuing the exposure to oxygen, the content of lipoperoxide began to decrease and reached a level lower than that of the control (at 24 h, $p < 0.01$; at 48 h, $p < 0.001$). After the exposure to oxygen for 12 h, the ERG amplitude began to decrease and became non-recordable after the exposure for 48 h (Figure 1). It should be noted that ERG began to decrease in accordance with the increase in lipoperoxide.

To observe the histological change in the retinal tissue, a light microscopic observation was made. Figure 2A shows the microphotograph of the retina of rabbit exposed to oxygen for 40 h. Comparison with the control (Figure 2B) obviously indicates pronounced degeneration in the visual cell layers in the case of oxygen exposure.

To measure the lipoperoxide formation in isolated retinas, the following experiments were carried out. Retinas were suspended in 0.9% NaCl and saturated with pure oxygen flowing from a pipette into the test tube for 45 min. The control experiment was done with argon flowing under the same conditions. These experiments were performed at room temperature and at ambient pressure. Lipoperoxide formation of retina suspension was measured by the above-mentioned TBA reaction. The amount of lipoperoxide in isolated retina exposed to oxygen increased approximately $2^1/_2$ times as compared with that of the control.

These results suggest the possibility that the formation of lipoperoxide in the retina is induced by high concentration of oxygen and the lipoperoxide denatures the associated proteins, resulting in an inability of retinal function as observed by ERG, and the change in structure as observed by light microscope.

Myopathic Changes at the End-Plate Region Induced by Neostigmine Methylsulfate

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Summary. Administration of large dose of neostigmine caused very quickly marked myopathic changes at the motor end-plate region. With continued injections, however, some recovery of the structural features did occur suggesting the reconstructive changes in the affected regions.

Neostigmine is a reversible anticholinesterase drug for the treatment of myasthenia gravis and other diseases. Previous studies have indicated that the repeated administration of this drug can produce some changes at the motor end-plates, such as atrophy of post-synaptic folds and the appearance of collapsed residues of pre-existing folds in widened synaptic spaces[3,4]. We report here that the repeated administration of large amounts of neostigmine methylsulfate produces not only changes at the motor end-plates, but also striking changes in the areas of muscle fibres adjacent to the motor end-plates.

Materials and methods. A total of 44 Sprague-Dawley rats weighing 200–250 g were used. 2 rats were injected s.c. with neostigmine methylsulfate at a dose of 0.06 mg/kg, 0.1 mg/kg, 0.25 mg/kg and 0.625 mg/kg (resolved in 1 ml saline solution), respectively. They were sacrificed 2 h after the injection. 10 rats received a single dose of 0.625 mg/kg and were sacrificed after 1 week and 2 weeks. 15 rats were given a daily injection with a dose of 0.625 mg/kg and sacrificed after 3 days, 1 week, 3 weeks, 6 weeks and 8 weeks.

In 6 other rats the right sciatic nerve was sectioned. From the 6th postoperative day, daily injections with a dose of 0.625 mg/kg were started and the rats were sacrificed after 2 days, 1 week and 3 weeks. 5 control rats were injected with equal volumes of physiological saline solution daily for 3 weeks and then sacrificed. Within 5 to 10 min following the injection, all of the

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animals began to display symptoms of muscle twitching, salivation, diarrhea and body tremor which sometimes lasted up to about 1 h.

While all rats given a single injection of 0.06, 0.1 and 0.25 mg/kg survived, 4 of the 10 rats injected with a single dose of 0.625 mg/kg died and 8 of the 15 rats injected repeatedly with 0.625 mg/kg died. 2 of the 6 denervated rats died. In all cases death was a result of the 1st injection and after the 2nd injection no further death was incurred, and with repeated injections these symptoms became progressively less marked.

Half of the soleus muscles of the surviving rats were either fixed with a solution containing 3% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.2) and postfixed in 1% osmium tetroxide, or fixed in 1% osmium tetroxide. Specimens were dehydrated and embedded in epoxy resin. Thick sections were stained with 1% toluidine blue solution for light microscopy. Sections showing silver-

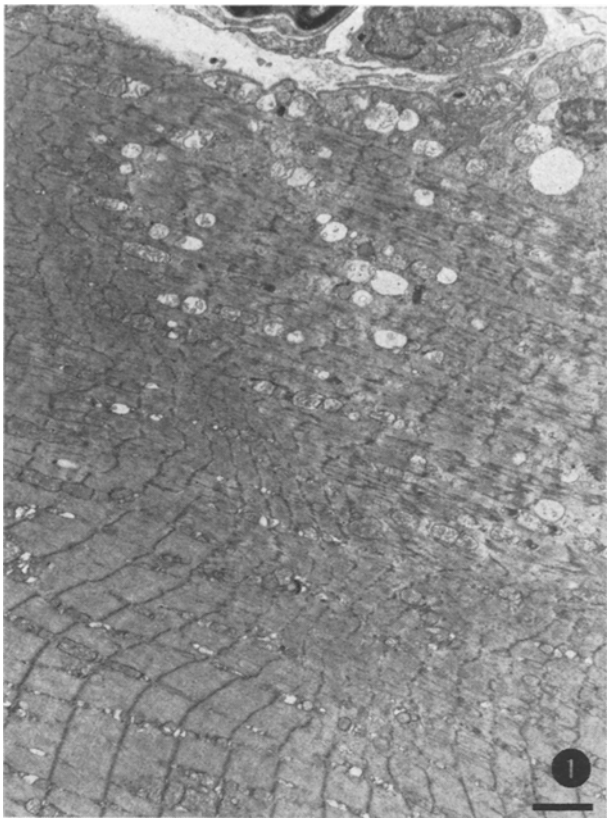

Fig. 1. A muscle fibre after 2 h of the injection with a dose of 0.625 mg/kg of neostigmine methylsulfate, which shows a striking focal change in the areas of the muscle fibre adjacent to a motor end-plate. Marked cytoarchitectural disorganization of the myofibrils is seen just beneath the end-plate. Fibrillar bundles surrounding the affected region are strongly contracted. Vacuoles, dense bodies and swollen mitochondria are visible in the sarcoplasm. The bar represents 2 μm.

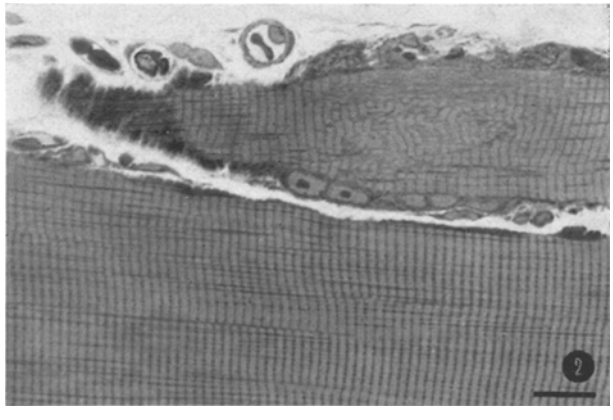

Fig. 2. A muscle fibre after 3 weeks of daily injections with a dose of 0.625 mg/kg of neostigmine methylsulfate, showing a focal lesion in the area of end-plate with light microscopy. The bar represents 20 μm.

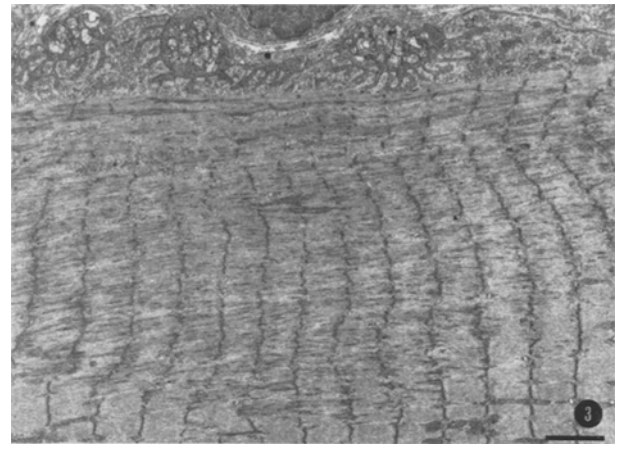

Fig. 3. After 3 weeks of daily injections with a dose of 0.625 mg/kg. Disorganized myofibrillar bundles with increased density of Z-like material are recognized in the sarcomeres. The bar represents 3 μm.

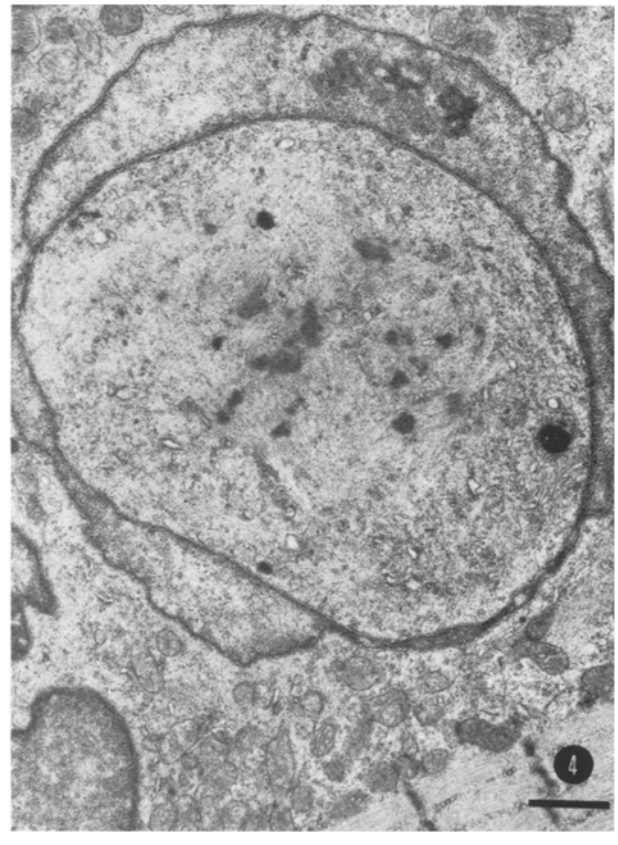

Fig. 4. After 3 weeks of daily injections with a dose of 0.625 mg/kg. Many membranous structures, ribosomal particles and disarranged fibrils with enlarged Z-lines are seen in the sarcoplasm encircled by an indented nucleus. The nucleus is filled with euchromatin-rich necleoplasm. The bar represents 6 μm.

gold interference colours were taken from selected areas of the blocks, stained with uranyl acetate and lead citrate, and examined under a JEM 7-A electron microscope.

The other half of the muscles were stained by the histochemical method of myofibrillar adenosine triphosphatase (ATPase) and reduced diphosphopyridine nucleotide dehydrogenase (DPNH).

Results. 1. Ultrastructural observations on soleus muscles after 2 h of the single injection with a dose of 0.625 mg/kg revealed dramatic localized lesions in the areas of muscle fibres adjacent to motor end-plates (Figure 1). In the areas just beneath the end-plates, myofibrils were stretched and severely disorganized and Z-like dense material was increased in the sarcomeres. In the sarcoplasm many dense bodies, vacuoles and swollen mitochondria were also observed. Myofibrillar bundles surrounding the severed regions were strongly contracted. No significant changes, except the swollen mitochondria, were found in the terminal axoplasm. In rats given only a single injection of the drug with a dose of 0.625 mg/kg, however, it became progressively difficult to find the focal lesions in the vicinity of the motor end-plates after the 2nd week. While the same changes were observed in rats receiving lesser dosages, these changes might be proportionate to the amount administered.

2. Ultrastructural observations on the muscles of the repeatedly injected rats demonstrated that sarcoplasmic changes such as vacuoles, dense bodies and swollen mitochondria disappeared from the first week on, whereas focal lesions showing increased density of Z-like material in the disorganized myofibrils could still be recognized up to the 8th week (Figures 2 and 3). From the 6th week, however, the affected regions were gradually decreased in number.

Lesions observed in the subsarcolemmal or central parts of the muscle fibre not far from the end-plate regions were found at all times. From the first week some of nuclei found in sole plate regions showed an apparent appearance of pyknotic processes, while many others were filled with euchromatin-rich nucleoplasm. Euchromatin-rich nuclei were more frequently found in sole plates than in control. Disarranged fibrils, many membranous structures and ribosomal particles were seen near these nuclei (Figure 4). The width of the primary synaptic clefts ranged from 500 Å to 900 Å, but secondary synaptic clefts were sometimes widened. Many dense granules with membranes were found in the synaptic spaces. Processes of Schwann cells interposing between axon terminals and synaptic folds were more frequently encountered than in control. The satellite cells were occasionally located near the sole plate regions.

3. In the soleus muscles, which were previously denervated and given injections with a dose of 0.625 mg/kg every day for 21 days, no focal changes as mentioned above were found in the muscle fibres of the end-plate regions but showed the denervation atrophy.

4. Histochemical staining of the ATPase and DPNH showed predominant atrophy in the type I fibres.

5. No significant changes, however, were found in the soleus muscle fibres of the control rats injected with physiological saline solution.

Discussion. In the experiment described neostigmine caused marked disorganization of myofibrils in skeletal muscle fibres at end-plate regions, even after the first injection. With repeated administration of the drug for 3 weeks, the whole soleus muscle decreased the weight at 30%, indicating muscular atrophy.

Euchromatin-rich nuclei found from the first week of the daily injections, together with many membranous structures and ribosomal particles, should reflect an elevated activity in the injured muscles. These features may suggest that a process of regeneration appeared in the sarcoplasm. After the daily injections, subsarcolemmal lesions or central core-like lesions were seen at all times, whereas those of the end-plate regions were less marked as time went by. With the single injection, on the other hand, focal changes at the end-plate regions were scarcely found in the muscle fibres after 2 weeks. In either case, it might be reasonable to assume that some reconstructive changes occurred in the affected muscle fibres.

Irreversible cholinesterase inhibitors such as DFP or Paraoxan containing organophosphorous compound can produce myopathy similar to this report[5–7]. Neostigmine inhibits the cholinesterase activity at the neuromuscular junction reversibly, and resultant excess amount of acetylcholine might mechanically or chemically play a role in the formation of these changes. This report will also present many problems concerning the histopathological features and treatment of myasthenic patients as well. Further investigations of this experiment are now underway.

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Laminar Acetylcholinesterase Localization in the Optic Tectum of Five Seawater Teleosts

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Summary. The histochemical localization of acetylcholinesterase in the optic tectum of seawater teleosts shows a characteristic laminar distribution which parallels the histological structure of the nervous centre. Significant differences have been observed between *Gobius* and the other 4 species of teleosts examined. It seems likely that cholinergic mechanisms play an important role in the function of teleost optic tectum.

The optic tectum usually reaches a noticeable degree of development in teleosts, playing a very important role in integration of visual input with other exteroceptive informations[1]. Wawrzyniak[2], using histochemical methods, observed a complex laminar distribution of acetylcholinesterase (AChE) in tectal layers of *Tinca*, while no positive reaction was achieved in *Cottus*. A recent study[3] on AChE localization in the optic tectum of 4 species of freshwater teleosts showed a similar pattern of distribution in 3 species while in the fourth, the catfish,

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