show neurones of a culture of rat spinal cord and cerebellum respectively, containing a high amount of SDH in their cell bodies and processes. 48 h after exposure to MMC (10^{-6} *M*), the enzymatic activity was markedly reduced (Figures 2 D and F). In contrast to this reduction of SDH-activity, it appeared that the content of AChE in cultured neurones is unaffected 48 h after exposure to MMC at a concentration of 10^{-6} *M*. Figure 2 B illustrates an example of a group of AChE-containing neurones of a spinal cord culture which was incubated with MMC (10^{-6} *M* for 48 h). AChE-activity was only reduced after 96 h of exposure, when extensive neuronal damage was observed.

Our histochemical studies, which are consistent with biochemical investigations by YOSHINO et al.[13] and TUNICLIFF and WOOD[26], revealed that SDH, an enzyme involved in metabolic processes, is more affected by MMC than AChE. There is at present no explanation for the different effects of MMC on these two enzymes.

Zusammenfassung. An Nervengewebskulturen menschlicher Foeten und neugeborener Ratten wurden Untersuchungen über die toxische Wirkung von Methylquecksilber (MMC) auf Neurone und Gliazellen im Rückenmark und Cerebellum durchgeführt. Die durch MMC erzeugte Degeneration zeigte eine deutliche Abhängigkeit von der Konzentration (10^{-6}–10^{-4} *M*) sowie von der Expositionszeit. Die histochemischen Befunde zeigen, dass MMC den Gehalt von Succinatdehydrogenase stark vermindert, die Acetylcholinesterase-Aktivität jedoch kaum beeinflusst.

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Effect of Scorpion Venom from *Tityus serrulatus* (Tityustoxin) on the Acetylcholine Release and Fine Structure of the Nerve Terminals

The finding that the venom of the South American scorpion, *Tityus serrulatus*, and a protein purified from it, Tityustoxin (TsTx), is able to liberate acetylcholine from several organs and tissues[1–4], suggests that TsTx may be a useful experimental tool to study mechanisms of acetylcholine release and storage.

This report describes effects of TsTx on the longitudinal strip of the guinea-pig ileum, a preparation very suitable for in vitro biochemical studies because of the relatively large production of acetylcholine confined exclusively to the nervous structures of the Auerbach's plexus[5], and on the mouse diaphragm, which is widely used in morphological studies of the neuromuscular junction. It has been found that TsTx increase the output of acetylcholine from the longitudinal strips of the smooth muscle. In the neuromuscular junction of the striated muscle, TsTx changes the structure of the nerve terminals, mainly the presynaptic vesicles and mitochondria.

The methods described by PATON and ZAR[5] were used for the preparation and mounting of longitudinal muscle strips of guinea-pig ileum and for the assay of acetylcholine. Extraction of acetylcholine was carried out as described previously[4]. Calciumfree media were made by omitting $CaCl_2$ and sodium-deficient Krebs solutions were prepared by substitution of sodium by equimolar solution of sucrose. The output of acetylcholine was calculated in nmoles of acetylcholine base/g wet tissue/min. *Tityus serrulatus* venom (LD_{50} i.p. route 62 µg/20 g mice) was kindly supplied by the Instituto Butantan. TsTx was purified by the method described by GOMEZ and DINIZ[6]. This method allowed preparation of a material which is homogeneous to polyacrylamide gel electrophoresis, and has an estimated LD_{50} of 1,6–2,7 µg/20 g mice.

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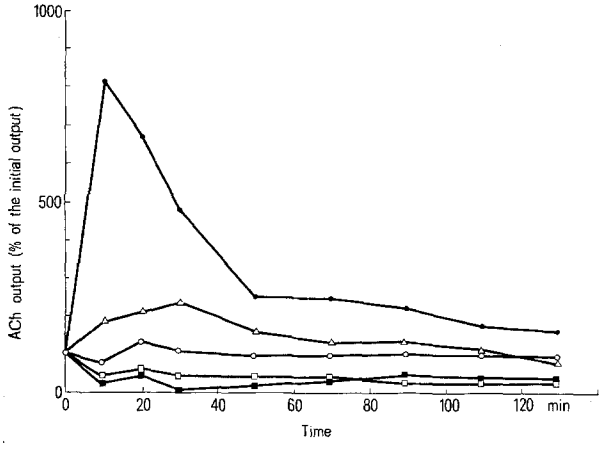

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Fig. 1. Effect of Tityustoxin on acetylcholine (Ach) output from eserinized longitudinal muscle strip of guinea-pig ileum. Initial rate of Ach output in presence of normal Krebs solution, 0.23 ± 0.03 nmoles/g/min taken as 100%. TsTx added as indicated in the text to reach a concentration of 12 µg/ml. ●—●, Ach release of a strip exposed continuously to TsTx in normal Krebs solution; △—△, rate of Ach release in a strip treated with TsTx in a Na-free solution, sucrose substitution; □—□, output of Ach produced by TsTx in strips bathed in a Ca-free Krebs solution; ■—■, TTx and TsTx added simultaneously to the normal bathing Krebs solution; ○—○, output of Ach of control strips suspended in normal Krebs solution in absence of TsTx. Output of control strips in Na- and Ca-free solutions or exposed to TTx in absence of TsTx are not shown as they did not differ significantly from the TsTx exposed strips in these conditions. Each point represents the acetylcholine output during the preceding period and was the mean of 2 experiments on the same achedule.

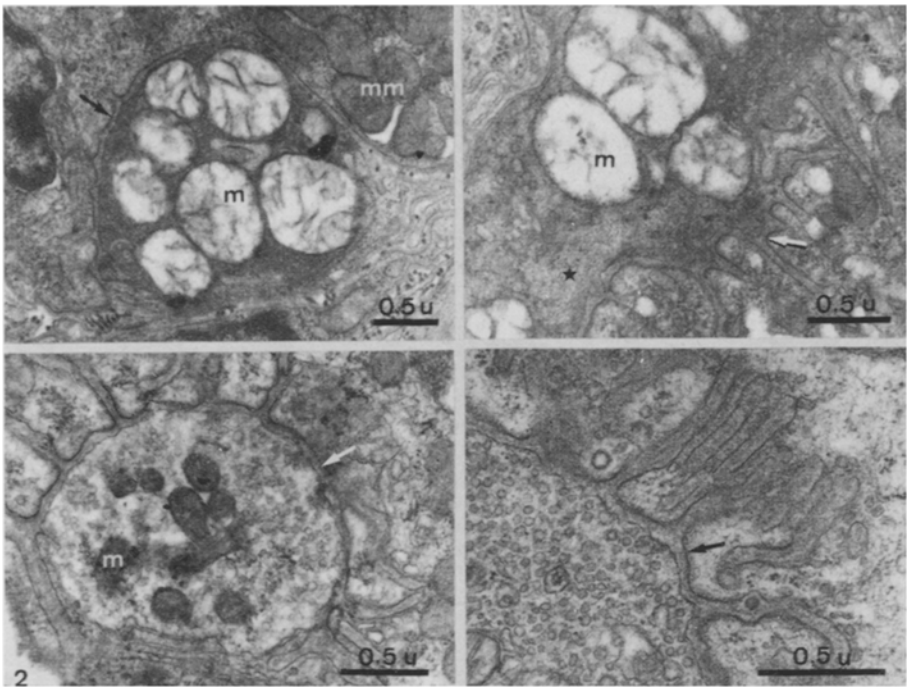

Fig. 2. Diaphragm motor end-plates of a mouse 60 min after injection with 8.0 μg of TsTx (upper figures) and of a normal mouse (lower figures). The mitochondria (m) are greatly swollen and the distribution of the synaptic vesicles is irregular in the treated animal. In the upper left figure the vesicles are pushed against the presynaptic membrane and in the upper right illustration there is an area (star) without vesicles. The normal mitochondria and the fairly uniform distribution of the synaptic vesicles may be appreciated in the lower figures.

A time course of the effects of Tityustoxin on the output of acetylcholine of longitudinal strips of the guinea-pig ileum under several conditions is illustrated in Figure 1. When a strip suspended in normal Krebs solution was exposed to TsTx in concentrations of $15–20 \times 10^{-6}$ g/ml, the output of acetylcholine rose abruptly 6–12-fold over the resting or control levels. In most of the experiments ,after about 1 h the output returned to a steady release of 2 or 3-fold above the controls, and was sustained for another 1 or 2 h. Washing of the preparation after a period of 5 or 10 min of exposure to TsTx did not change the pattern of release. This result suggests a strong binding of the toxin to the nervous structures. Omission of calcium or sodium from the incubation media prevents the effect of TsTx on acetylcholine release. Tetrodotoxin (TTx) (5×10^{-7} g/ml), a substance known to interfere with the inward flux of Na ions[7,8], blocks the effect of TsTx when both toxins are simultaneously added to the organ bath, or after 5 or 10 min of exposure of the strip to TsTx.

For ultrastructural studies, 5 mice were injected i.p. with a solution of purified TsTx (4–14 μg per mouse) and 3 intact animals were used as controls. The animals were sacrified 30 to 190 min after the administration of the toxin and the diaphragms immediately removed and fixed. Routine double fixation (glutaraldehyde followed by osmium tetroxide) and Palade's fixative were used. The material was embedded in Araldite, sectioned and stained with uranyl acetate and lead citrate. An AEI-EM6B electron microscope was employed.

The axons and neuromuscular junctions of the diaphragm of mice treated with TsTx display striking pathological changes. The axons situated close to the nerve terminals show degenerations consisting of shrinkage and gross vacuolizations of the axoplasm. The mitochondria of the nerve ending are greatly swollen and fill most of the presynaptic apparatus. The synaptic vesicles are irregular in size and density and have an abnormal distribution. The synaptic cleft and the postsynaptic part of the motor end-plates are normal (Figure 2).

It is interesting to note that the venom of another arachnid, the black widow spider, also depletes the nerve terminals of their vesicles[9,10] and changes the morphology of the axons in a similar way.

Although the synaptic vesicles are believed to be related directly to acetylcholine release[9–13], it is not easy to establish a clear connection between our morphological findings and the release process. It can only be said that the ultrastructural alterations of the motor end-plate of the diaphragm of mice injected with TsTx lend support to the idea that this toxin changes the permeability properties of presynaptic membranes.

Zusammenfassung. Nachweis, dass ein Giftstoff des südamerikanischen Skorpions *Tityus serrulatus* auch in vitro am isolierten Meerschweinchendarm zu stark erhöhtem Acetylcholinausstoss führt der bei Kalziumentzug und Blockade des Natriumeinstroms durch Tetrodotoxin blockiert werden kann. Elektronenoptisch sind im Mäusezwerchfellpräparat nur im präsynaptischen Gebiet pathologische Veränderungen zu sehen.

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