Condition of the firebrat ovarioles affected by vapours of *Acorus calamus*

Dose	Ovarioles[a]	Condition of the ovarioles after days of exposition to the vapours		Remarks
		16 days[c]	25 days[d]	
0 (pure acetone)	Germarium	Finger like. 20 oögonia, numerous prefollicular cells, with 8 to 10 young oöcytes.	The same picture, new oöcytes ripening	Females moulted twice. Egg laying 3 times, about 30 eggs from each female
	Previtellarium	More than 10 oöcytes. Start of follicular epithelium formation in proximal part.		
	Vitellarium	In the state of formation.		
		16 days[c] (Figure 1)	25 days[d]	
3 ml/1000 ppm	Germarium	Rounded shape, undifferentiated oögonia no young oöcytes	In 2 ov. completely destroyed, in others oögonia and prefol. cells with pycnotic nuclei.	Egg laying once only, 18 eggs were layed in average from each female, of which about 10% could not hatch
	Previtellarium	Present only in 5 of 10 ovarioles	Malformations found in 4 ovarioles. Granulation in all oöcytes, some are completely destroyed.	
	Vitellarium	Not developed, cap-like at the proximal part of the ovariole	Not developed.	
		6 days[b] (Figure 2)	22 days[d] (Figure 3)	
9 ml/1000 ppm	Germarium	Oval or rounded in shape, highly hypertrophied, filled with oögonia; part of pref. nuclei pycnotized; no young oöcytes	Not developed, empty tubes with chromatin granules	No eggs. Distal part of 1 ovariole after detaching formed rounded body with 8 previtellar oöcytes and rests of germarium
	Previtellarium	Thread-like, numerous prefollicular cells no more than 2 oöcytes in 1 ovariole.	Very thin with 1 or 2 oöcyte nuclei in the state of resorption.	
	Vitellarium	Reduced, being formed in 4 of 10 ovarioles with remnants of resorbed chorionized eggs, no vitellogenesis.	Even the tunica propria after interrupted, disconnecting thus 2 ends of 10 ovarioles.	
		27 days[d] (Figure 4)		
13 ml/1000 ppm	Germarium	Reduced entirely in 3 ovarioles where it is empty, in others pycnotic nuclei and chromatine granules.		
	Previtellarium	Without follicular cells. 1 oöcyte present in only 1 of the ovarioles.		
	Vitellarium	Does not exist the whole length of ovarioles; not more than twice the length of spermetheca.		

[a] Ovary of panoistic type, with 10 ovarioles, 5 in 1 bunch. [b] 1st, [c] 2nd, [d] 3rd inter moulting period. ppm = Part per million solution in acetone.

Zusammenfassung. Eine Behandlung der Weibchen von *Thermobia domestica* mit den Dämpfen eines Extrakts der Wasserpflanze *Acorus calamus* L. bewirkte dauernde Sterilität, da die Oogonien und präfollikulären Zellen in den Germarien und die follikulären Zellen und Oozyten in den Pävitellarien zerstört wurden. In manchen Fällen wurde die Tunica propria zerstört, was zur Unterbrechung der Ovariolen führte.

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The Effects of Methyl Mercury on Morphological and Histochemical Properties of Human and Rat Spinal Cord and Cerebellum in Tissue Culture

It has been shown by several laboratories that alkyl mercury compounds, in particular methyl mercury, are by several orders of magnitude more toxic than other mercury compounds, and that methyl mercury primarily affects the central nervous system (CNS)[1–3]. Furthermore, observations of mercury poisoning in human subjects have revealed that the fetal CNS is more sensitive to methyl mercury than the adult brain[4]. The neurotoxic effects of this compound have been investigated extensively in clinical cases[5–7] and in experimental studies on animal CNS in vivo and in vitro[3,8–12]. It was observed that methyl mercury produces extensive damage to neurones, neuroglia and nerve fibres. Granule cells of the cerebellum and dorsal root ganglion cells appear to be more sensitive to the toxic effect than neurones of other regions of the CNS[7,8,9,13]. Since the method of tissue culture is a useful tool to study the effects of toxins on the mammalian CNS and especially on the human CNS, we have

investigated the action of methyl mercury on morphological and histochemical properties of cultured spinal cord and cerebellum of human fetuses and newborn rats.

The cultures were prepared from spinal cord and cerebellum of human fetuses (7–8 weeks in utero) and of newborn rats, and grown on collagen-coated coverslips in the Maximov assembly at 35 °C[14–16]. After 14 to 22 days in vitro, methyl mercury chloride (MMC) was added to the nutrient medium in concentrations of 10^{-4} to 10^{-6} *M*. The cultures were observed daily on a reverse microscope using phase contrast optics. A series of cultures was stained for acetylcholinesterase (AChE) and succinic dehydrogenase (SDH) using the histochemical methods of KARNOVSKY and ROOTS[17] modified by EL BADAWI and SCHENK[18] and of NACHLAS et al.[19] respectively (for details see[20–22]).

The outgrowth pattern of the various cell types in cultures from human and rat spinal cord and cerebellum has been described previously[15, 16, 23–25]. The extent and time of development of the neurotoxic effects of MMC were clearly dependent on the concentration of the compound in the nutrient medium. 6 h after addition of MMC in a concentration of 10^{-6} *M*, the glial cells in the outgrowth zone showed granulation in the cell body and processes (Figure 1 A). After 24 h the granularity increased and the granules became coarser. Furthermore, the processes of the glial cells started to retract (Figure 1 B). After 48 h, almost all glial processes had retracted and only the cell bodies adhered to the collagen substrate (Figure 1 C). After 5–7 days, the cytoplasm of the astrocytes showed vacuoles and the cells degenerated. After exposure of the cultures to a concentration of 10^{-4} *M*, vacuoles in the cell body and a watery space in the perinuclear region of the glial cells were observed already after 6 h (Figure 1 D). Cell death occurred between 24 and 48 h.

Neurones of spinal cord cultures of human fetuses, as well as of newborn rats, which were exposed to MMC at a concentration of 10^{-6} *M* showed the first degenerative signs after approximately 8 h. The cell bodies, the dendrites and axons revealed swelling and granulations (Figure 1 F). At this stage, the nucleus of most neurones still remained in its central position. After 48 h, there was an increase of granularity in the cell body and processes and the nucleus usually now had an excentric position (Figure 1 G). Between 6 and 10 days after exposure to MMC, the neurones showed progressive signs of degeneration with a great number of vacuoles. After addition of MMC in a concentration of 10^{-4} *M*, it was observed that there was already an extensive neuronal damage after 6 h (Figure 1 H).

In cerebellar cultures of both human fetuses and newborn rats, the first neurones to show degenerative signs after exposure to MMC were granule cells[8, 11]. Figure 1 I illustrates part of a human cerebellar culture, 8 h after incubation with MMC at a concentration of 10^{-6} *M*. In contrast to Purkinje cells, which reveal only a slight swelling of the cell body, most granule cells already show signs of degeneration at this time. After 20 h of exposure, a great number of granule cells have degenerated with vacuoles in their cytoplasma, whereas Purkinje cells only showed slight granulations in the cell body (Figure 1 J). After 48 h, almost all granule cells have completely degenerated but Purkinje cells have only increased granulation in the soma and processes at this stage (Figure 1 K). Complete degeneration of Purkinje cells occurred after 7–10 days. Figure 1 L illustrates a cerebellar culture, 48 h after addition of MMC at a concentration of 10^{-5} *M*. The Purkinje cells contain coarse granules in their cell bodies and processes but they are still quite well preserved, whereas the majority of granule cells have degenerated (arrow).

Biochemical studies have demonstrated that the synthesis of several enzymes in the CNS are inhibited by mercury compounds[10, 13, 26]. The activity of succinic dehydrogenase (SDH), an enzyme involved in the citric acid cycle, was significantly decreased after treatment of animals with methyl mercury thioacetamide[13]. In contrast, acetylcholinesterase (AChE), an enzyme which plays a rôle in synaptic transmission, seems to be unaffected by methyl mercury compounds[26]. The activity of SDH and AChE was studied histochemically in a series of cultures of rat and human spinal cord and cerebellum exposed to MMC 10^{-6} *M*. As was observed with biochemical techniques[13], the activity of SDH was markedly decreased after exposure of the cultures to MMC. Figures 2 C and E

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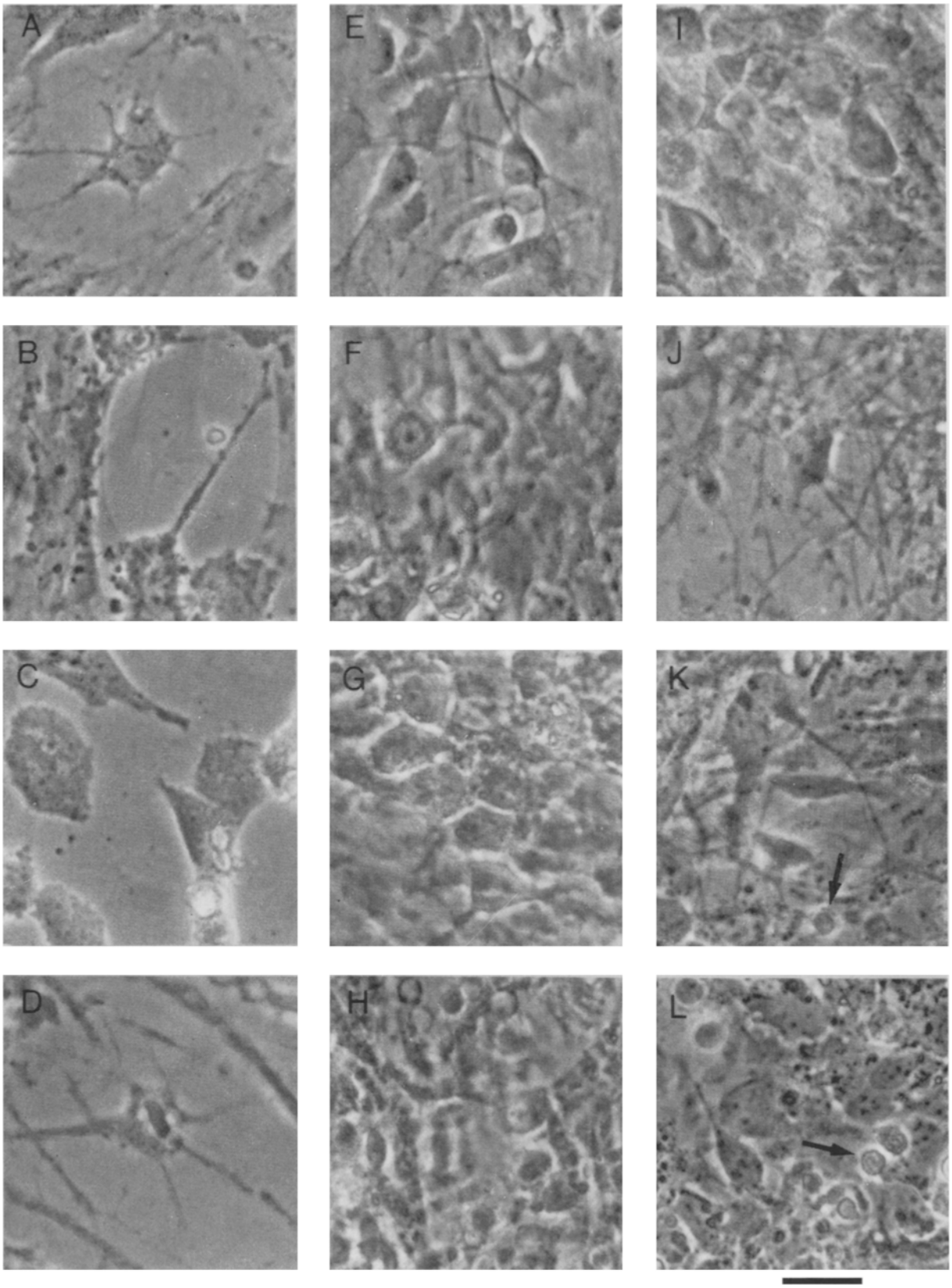

Fig. 1. Effects of methyl mercury on morphological properties of neurones and glial cells of human and rat spinal cord and cerebellum in tissue culture. (A–C) Degenerative changes of glial cells of fetal human spinal cord cultures 6 (A), 24 (B) and 48 (C) h after addition of 10^{-6} *M* MMC to the nutrient medium. (D) 6 h after addition of MMC at a concentration of 10^{-4} *M*. (E) Neurones of a rat spinal cord culture (22 days in vitro) before exposure to MMC. (F, G) Rat spinal cord cultures (22 days in vitro) 6 (F) and 48 (G) h after exposure to MMC 10^{-6} *M*. The neurones reveal signs of degeneration such as swelling of the cell body, granulation, excentric localization of the nucleus. (H) Marked damage of neurones and glial cells 6 h after addition of MMC in a concentration of 10^{-4} *M* (rat spinal cord culture, 17 days in vitro). (I–K) Human cerebellar cultures (fetus 8 weeks in utero, 17 days in vitro) after exposure to 10^{-6} *M* MMC for 8 (I), 20 (J) and 48 (K) h. Note swelling of Purkinje cells after 8 h (I). After 20 (J) and 48 (K) h of exposure, increasing granulation in Purkinje cells was observed whereas granule cells have almost completely degenerated (K, arrow). (L) Cerebellar culture after exposure to MMC at a concentration of 10^{-5} *M* for 48 h. Note marked granulation in Purkinje cells and degeneration of the majority of granule cells (L, arrow). Phase contrast pictures, Bar: 20 μm.

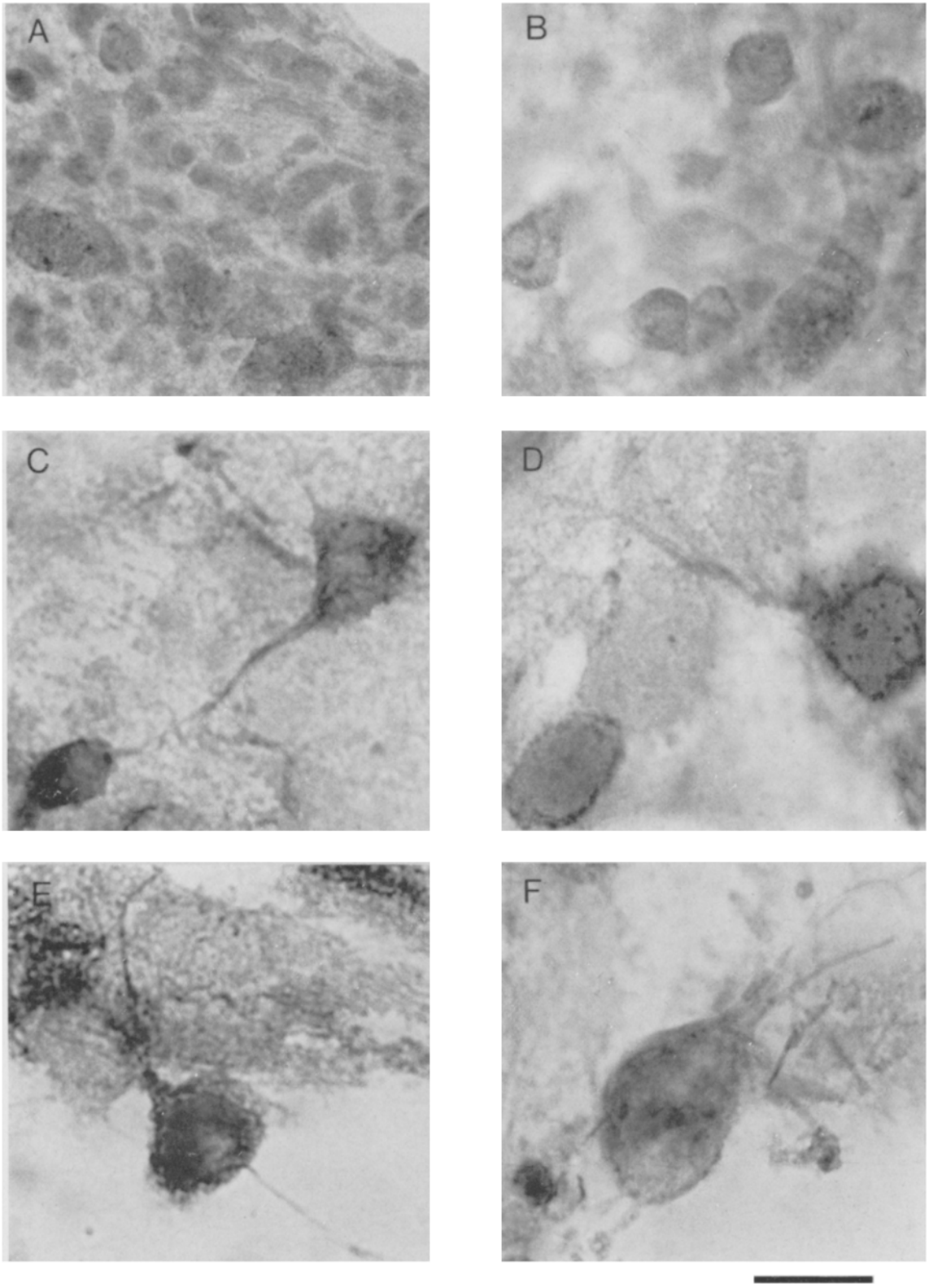

Fig. 2. Effects of methyl mercury on histochemical properties of rat spinal and cerebellar neurones in tissue culture. (A) Group of neurones in a rat spinal cord culture (27 days in vitro) stained for AChE. (B) Exposure of spinal cord cultures to MMC, 10^{-6} *M* for 48 h causes no significant change of AChE-content. (C, E) Rat spinal cord (C) and cerebellar cultures (E) (18 days in vitro) stained for SDH. (D, F) Marked decrease of SDH-activity in spinal neurones (D) and in Purkinje cells (F) after addition of MMC, 10^{-6} *M* for 48 h. Bar: 20 µm.

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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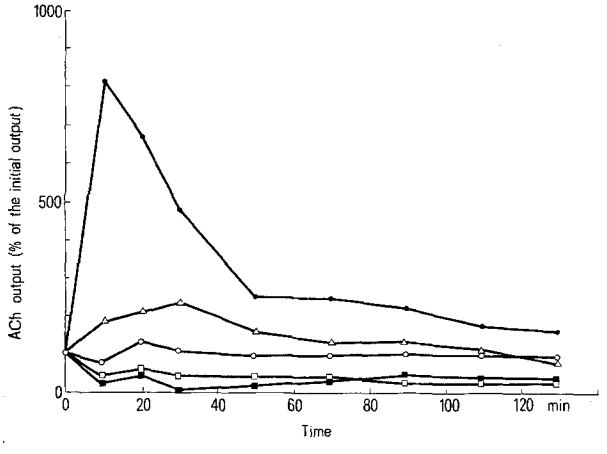

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Fig. 1. Effect of Tityustoxin on acetylcholine (Ach) output from eserinized longitudinal muscle strip of guinea-pig ileum. Initial rate of Ach output in presence of normal Krebs solution, 0.23 ± 0.03 nmoles/g/min taken as 100%. TsTx added as indicated 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.