Analysis calculated for $C_{28}H_{30}O_{13}$ (M.W. 574.5) was C, 58.53; H, 5.26; O, 36.21. Found: C, 58.31; H, 5.52; O, 35.89.

Antibiotic U-20,661 inhibits gram-positive bacteria in vitro (Table II) but is inactive against gram-negative bacteria when assayed in a two-fold broth dilution test[3]. No antibacterial activity was observed in mice experimentally infected with *Streptococcus hemolyticus*[3] when treated s.c. at the maximum tolerated dose. No blood serum levels were demonstrated at the same dose and administration route. The antibiotic was inactive against T_6-phage in *E. coli* assayed according to ADAMS[4].

Antibiotic U-20,661 inhibits the growth of KB-cells yielding an ID_{50} (50% inhibition of protein synthesis) of 1.6 μg/ml using the assay system described by SMITH et al.[5]. The antibiotic is, therefore, extremely cytotoxic against mammalian cells grown in vitro. Conversely, the compound is remarkably non-toxic in mice. Maximum tolerated doses were 800 mg/kg day orally, 400 mg/kg day s.c. or 100 mg/kg day when administered i.p. The acute LD_{50} (i.p.) was 562 mg/kg in mice.

Zusammenfassung. Ein neues Antibiotikum, U-20661, wurde aus der Kulturflüssigkeit eines Streptomycetenstammes isoliert, welches in vitro nur gegen grampositive Bakterien wirksam ist. Die Substanz ist extrem cytotoxisch in Gewebekulturen, aber ungiftig für Mäuse.

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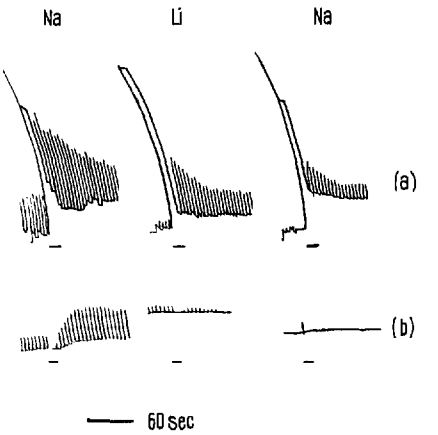
The Effect of Lithium Ions on the Post-Tetanic Potentiation of Neuro-Muscular Transmission

In partially curarized nerve-muscle preparations, neuro-muscular transmission recovers after a period of repetitive activity[1]. This recovery seems to be due to increased transmitter liberation following tetanus[1,2]. It has been suggested that the increase is caused by changes in action potential associated with the post-tetanic hyperpolarization[3]. Since the post-tetanic hyperpolarization is greatly reduced or abolished when the extracellular Na is replaced by Li[4,5] the effect of Li on neuromuscular transmission and on post-tetanic potentiation was studied.

Isotonic contractions and nerve action potentials were recorded from the guinea-pig hemidiaphragm-phrenic nerve preparations[6] suspended in Krebs, or modified Krebs solution, to which $3 \cdot 10^{-4} M\ L^{-1}$ choline had been added. The solutions were equilibrated with 95% O_2/5% CO_2 and kept at 35–37 °C. The nerve was stimulated so that maximal muscle twitches were obtained and D-tubocurarine or Mg was added until the muscle twitch was reduced to about $^1/_5$ of its maximal amplitude. Stimulation was maintained at a rate of 0.2 shocks/sec and was then increased for 10 sec to 200 shocks/sec. After the period of tetanic stimulation, the muscle contractions were several times larger than before the tetanus. Further stimulation at 0.2 shocks/sec often gave a few contractions of increasing amplitude. The amplitude of the subsequent contractions gradually returned to the pre-tetanic level within 20–60 sec (Figure). In preparations blocked with D-tubocurarine, the muscle did not contract during the tetanus; in Mg-blocked preparations tetanic muscle contractions were observed. Direct stimulation of the muscle in these preparations showed that the muscle twitches were not increased after the tetanus.

When the Krebs solution was replaced by a solution in which Li had been substituted for Na, neuro-muscular transmission in non-curarized preparations was maintained for about 8 min. The muscle twitches then decreased and neuro-muscular block occurred after 15 min.

Post-tetanic potentiation was found in curarized preparations kept in Li-solution. The potentiation was about the same as in Na-solution when the preparations



Effect of Li on post-tetanic potentiation in partially curarized hemidiaphragm-phrenic nerve preparation. Nerves were stimulated at 0.2/sec and stimulation frequency increased to 200/sec for 10 sec (—). Left-hand records were taken with preparations in Na, centre records in Li, and right-hand records again in Na solution. In experiment (a) solutions contained $1.1 \cdot 10^{-5} M$ Mg and pre-tetanic exposure to Li was 5 min; in experiment (b) the preparation was curarized with $1.5 \cdot 10^{-9} M$ D-tubocurarine and exposure to Li was 10 min at 37 °C.

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were left for 4 min in Li (Figure, a). This time-period was found, in control experiments with sucrose-Krebs, to be sufficient for exchanging more than 90% of the extracellular fluid. Potentiation also occurred when the preparations were kept for 5–8 min in Li: on returning to Na there was then an increase in the amplitude of the contractions prior to tetanic stimulation. When the preparations were kept for about 10 min in Li, the post-tetanic potentiation was abolished (Figure, b), on still longer exposure to Li, neuro-muscular block was found. In Na the block disappeared slowly over a few hours.

The observation of post-tetanic potentiation in Li suggests that the potentiation is not caused by post-tetanic hyperpolarization. The post-tetanic hyperpolarization in non-myelinated fibres is greatly reduced after short exposure to Li and abolished after 4–5 min in Li[4]. Similar effects are seen in myelinated fibres where studies of the Li effects[7,8] showed that an initial short phase of hyperpolarization may still be found after exposure to Li for some minutes, whereas the long-lasting hyperpolarization, which follows about the same time course as the post-tetanic potentiation, is abolished as the extracellular Na is replaced by Li. The present experiments also exclude the hypothesis that post-tetanic facilitation is caused by accumulation of intracellular Na[9]. Our conclusions are similar to those of HUBBARD and GAGE[10] who used metabolic inhibitors, cardiac glycosides and reduced Na concentrations for inhibiting the post-tetanic potentiation.

Zusammenfassung. Am partiell curarisierten Zwerchfell-Phrenicus-Präparat des Meerschweinchens wurde gefunden, dass die post-tetanische Fazilitation auch in Li-haltiger Lösung auftritt. Die Fazilitation scheint nicht durch post-tetanische Hyperpolarisation entstanden zu sein, da diese nach Li-Applikation ausbleibt.

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Nucleolar Changes in Spinal Ganglion Neurons During the Course of Axon Regeneration

The increase of nucleolar volume in neurons after axonal section has been known for a long time. However, the changes which occur in this process have not been studied in the light of some new concepts of nucleolar structure. It is generally accepted[1] that the nucleolus is made up by a filament, the nucleolonema, which is embedded in a matrix or pars amorpha. The nucleolonema of rat spinal ganglion neurons was easily demonstrated with silver impregnation techniques[2] (Figure 2) or by phase contrast microscopy in vivo following a procedure for cell isolation[3] (Figure 1). Nucleolar changes were studied after crushing the left sciatic nerves of 20 rats of our stock at the proximal third of the femur. The animals were sacrificed after 1 to 15, 20, 30, 40, 50 and 60 days. The left (operated) and right (control) spinal ganglia were carefully dissected and fixed in ethanol-acetic acid, 3:1. After paraffin embedding 5 μ sections were stained with buffered thionin[4]. This method demonstrated the nucleolonema quite well and it appeared like a network instead of a clear-cut filament (Figure 3).

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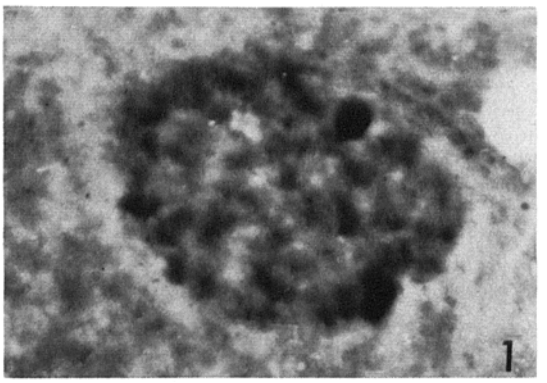

Fig. 1. Squash preparation of a spinal ganglion neuron isolated in vivo observed with the phase contrast microscope. The helicoidal filament contrasts neatly against a clear background. ×3000.

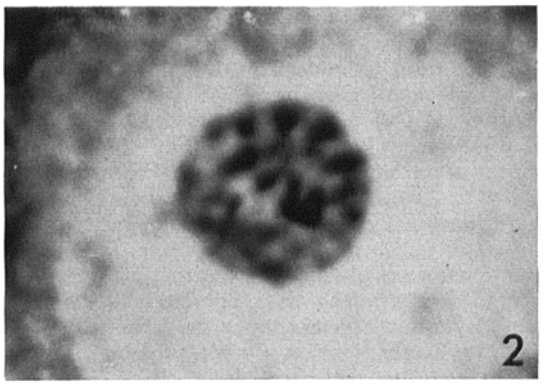

Fig. 2. Silver impregnation of a spinal ganglion neuron of the rat. The nucleolonema appears as a heavily impregnated filament. ×3000.