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The Effect of Puromycin on Neuromuscular Transmission

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WULFF, V. J. *The effect of puromycin on neuromuscular transmission.* PHARMAC. BIOCHEM. BEHAV. 1(2) 177–182, 1973.–Twitch tensions of directly and indirectly stimulated frog sartorius muscles and the mechanical responses to exogenous acetylcholine were recorded in the presence and absence of puromycin. This drug reduced the response to acetylcholine and indirect stimulation but did not affect the response of directly stimulated frog sartorius muscles. These effects were reversible. Summed motor end plate potentials of frog sartorius muscles were recorded in the absence and presence of puromycin and acetoxycycloheximide. Puromycin reduced the magnitude of the motor end plate potential but acetoxycycloheximide did not. This effect of puromycin was only partially reversible. The effects of curare on twitch tension of indirectly stimulated muscles and on the motor end plate potential were compared with the effects of puromycin.

Frog Sartorius muscle Twitch tension Motor end plate potential Puromycin Acetoxycycloheximide

THE EXPERIMENTS described below were undertaken to determine if the antibiotic, puromycin, which has a pronounced inhibitory effect on protein biosynthesis [22] would alter the properties of a synaptic system, specifically, the myoneural junctions of the frog sartorius muscle. Early in this investigation it appeared unlikely that the dramatic and immediate effects of puromycin on the response characterisitcs of the sartorius muscle nerve preparation could be attributed to interference with protein biosynthesis but, rather, that these effects represented a curare like action of the drug. In this respect, puromycin mimics the effect of neomycin, kanamycin and streptomycin [6].

Nevertheless, it was decided to continue this investigation in view of the reported interference of puromycin with the retention of learned responses in mice [13, 14, 16, 18] and in goldfish [1, 2, 3] an interference which was attributed to the inhibition of protein synthesis by puromycin.

Experiments in learning and retention utilizing cycloheximide and acetoxycycloheximide, substances which also inhibit protein synthesis [11,23] have produced results at variance with those obtained with puromycin. In mice, Flexner and co-workers [15,17] found no impairment of retention of learned responses with acetoxycycloheximide, although the inhibition of protein synthesis was severe and long lasting. Moreover, they demonstrated that acetoxycycloheximde, when injected together with puromycin, antagonized the amnesic effect of the latter. Similar observations were made by Barondes and Cohen [5] but more recently Cohen and Barondes [9] demonstrated an impairment of learning following injection of acetoxycycloheximide and presented evidence that puromycin injected into mouse brain promotes seizure activity in the hippocampus. In goldfish, intracranial injection of acetoxycycloheximide interfered with retention of a learned response but to a lesser extent than puromycin, despite a more severe reduction in the incorporation of radio-leucine into brain protein by acetoxycycloheximide [7]. These authors also report that puromycin at a low dose (50 μg) produced inhibition of radio-leucine incorporation for two hours but had no effect on retention of a learned response.

These observations indicate that puromycin inhibits the incorporation of radio-amino acids into brain protein but also exerts other effects on neuronal activity which may be responsible for the amnesia effect. It is likely that the effects of puromycin on the cholinergic synaptic system described below may also occur on cholinergic synaptic systems which presumably exist in the central nervous system [10, 19, 20, 21, 24].

METHOD

The experiments were performed on sartorius muscle and muscle-nerve preparations obtained from *Rana* pipiens. One hundred fifty one experiments were performed in this study, most of them utilizing paired muscle or muscle nerve preparations from the same animal. Puromycin dihydrochloride was obtained from Nutritional Biochemicals Corporation, Cleveland; acetoxycycloheximide was obtained from the Charles Pfizer Company, Maywood, New Jersey.

Two Ringer's solutions were used. All experiments in which twitch tensions were monitored utilized a bicarbonate buffered Ringer's equilibrated with a 95% oxygen, 5% CO_2 gas mixture which had the following composition

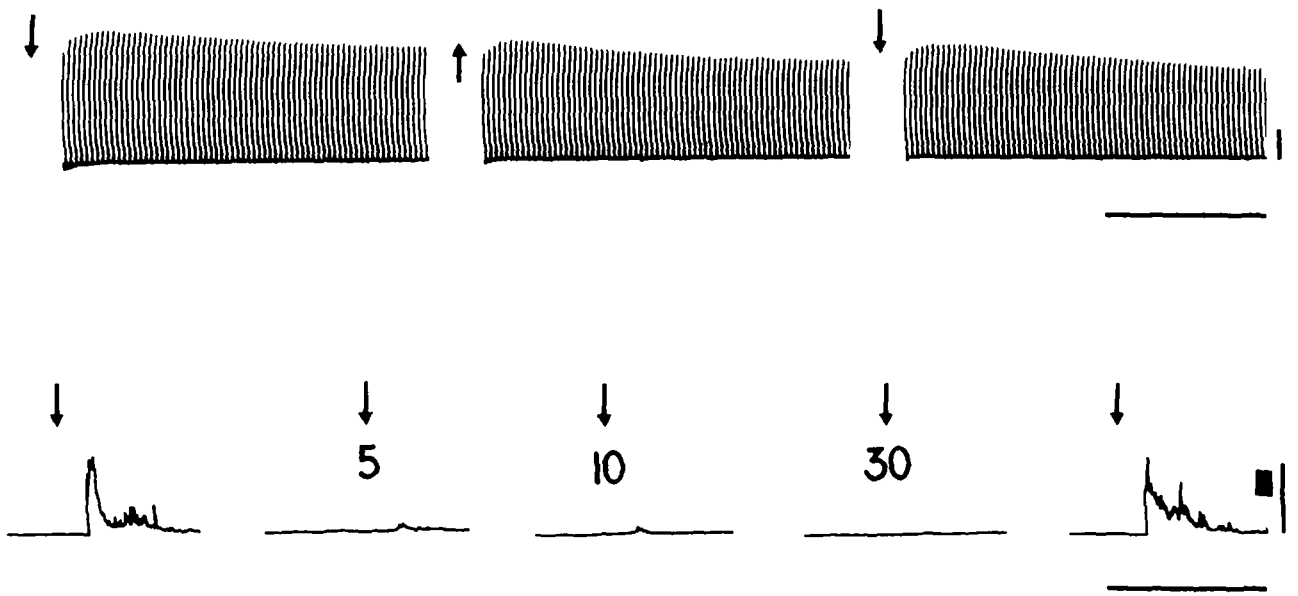

FIG. 1. Upper: Records of the twitch tension of an indirectly stimulated sartorius muscle in the presence (arrow down) and absence (arrow up) of puromycin (0.39 mM). Stimulation was interrupted for 30 sec when the bathing solution was changed. Lower: Records of the contraction of a sartorius muscle to exogenous acetylcholine (0.055 mM) in the absence (extreme right and left) and in the presence of puromycin (0.39 mM). The arrows indicate the time of addition of acetylcholine and the numbers indicate elapsed time in seconds between the addition of puromycin and acetylcholine. Calibrations, 1 min and 1 g.

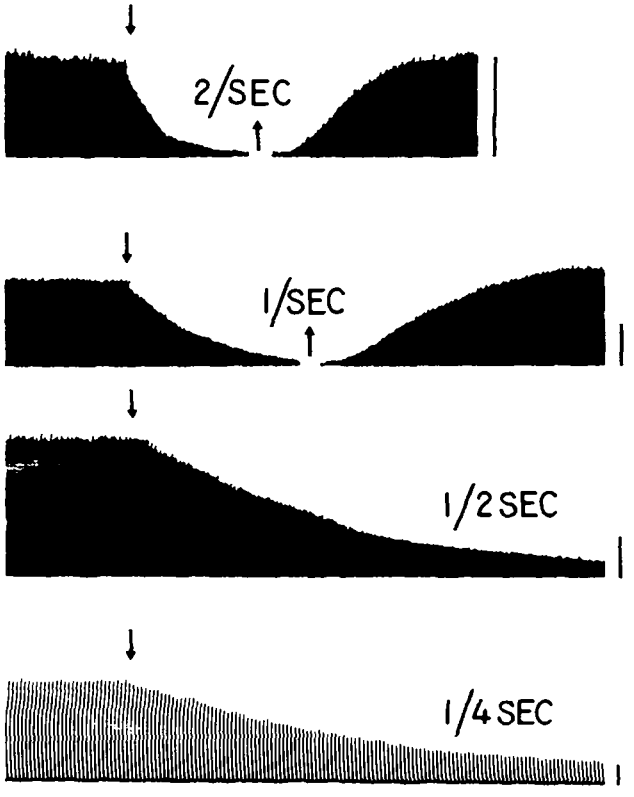

FIG. 2. Records of the twitch tension of a frog sartorius muscle stimulated indirectly at the indicated frequencies. The downwardly directed arrows indicate the time of addition of puromycin (bath concentration, 0.39 mM) and the upwardly directed arrows indicate its removal. Recovery of the mechanical response at lower frequencies was omitted because of the length of the records. Calibrations, 1 min and 1 g.

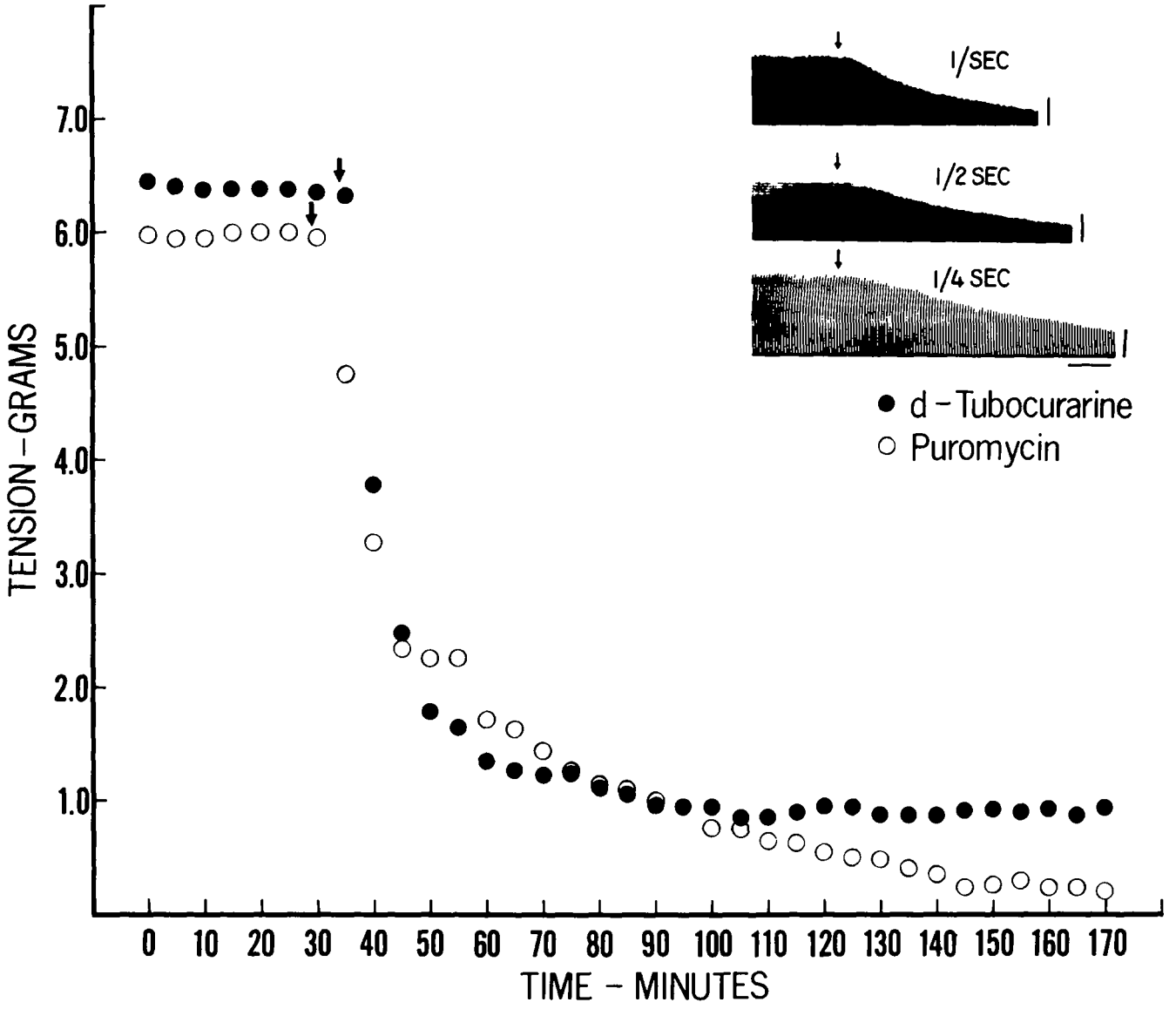

FIG. 3. The twitch tensions of indirectly stimulated paired sartorius muscles of the frog before and after one was exposed to puromycin (0.39 mM) and the other to *d*-tubocurarine (4μM). The arrows indicate the time of application and the frequency of stimulation was 1/5 min. Inset: the effect of frequency of stimulation on the rate of development of *d*-tubocurarine induced inhibition. Calibrations, 1min and 1g.

(mM): NaCl, 114; KCl, 1.9; $CaCl_2$, 1.8; $NaHCO_3$, 4.8; NaH_2PO_4, 0.1; glucose, 11.1. All experiments in which end plate potentials were monitored utilized a phosphate buffered Ringer's of the following composition (mM): NaCl, 117.8; KCl, 2.0; $CaCl_2$, 1.7; NaH_2PO_4, 2.54. To eliminate muscle action potentials $MgCl_2$ replaced NaCl in amounts sufficient to obtain the required anesthesia [8]. The pH of solutions in contact with the preparations was between 6.8 and 7.2. It was necessary to neutralize the puromycin solutions.

Muscle twitch tensions were monitored by force displacement transducers (Grass model FT .03C) and recorded with a polygraph (Grass model 5A). The vertically mounted muscles were immersed in a 36 ml bath through which a gas mixture (95% O_2, 5% CO_2) was bubbled continuously. When indirectly stimulated, pulses from a generator (Grass model S4) were delivered to the sartorius muscle nerve lying in a tunnel through a lucite block containing two Ag-AgCl coated plates, and the entire assembly was submerged to eliminate dessication of the nerve. When directly stimulated, the pulses were delivered to the muscle via two Ag-AgCl electrodes imbedded in agar within glass tubes, one making contact with the pelvic end of the muscle in moist air and the second immersed in the bath.

Motor end plate potentials from the sartorius muscle were recorded via Ag-AgCl agar wick electrodes after the method of Fatt [12] and the nerves were stimulated via a submerged tunnel electrode. The potentials were amplified and displayed on a cathode ray oscilloscope (Tektronix 502) and recorded photographically (Grass Kymograph

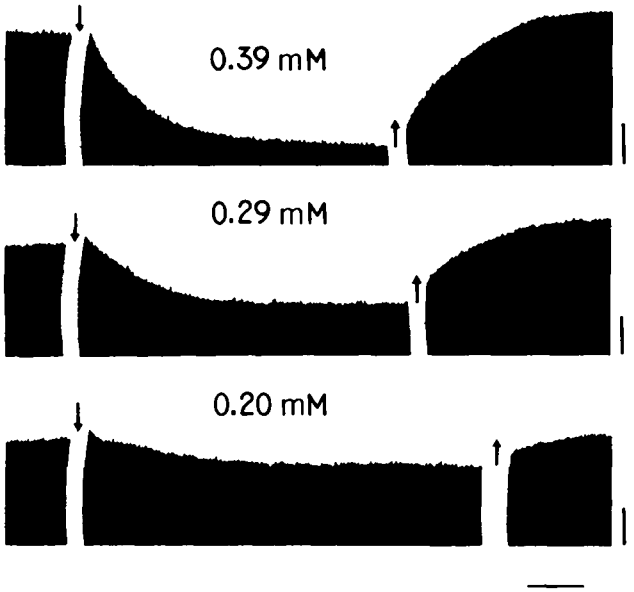

FIG. 4. Records of the twitch tension of an indirectly stimulated frog sartorius muscle (frequency, 1/sec) in the presence of puromycin at the given concentrations. The arrows indicate the time of addition and removal of puromycin and the calibration marks indicate 1 min and 1 g.

camera). In these experiments, the liquid level was adjusted for maximal amplitude of response generally within 1 cm below the pelvic end of the sartorius muscle.

RESULTS AND DISCUSSION

The addition of puromycin to the solution bathing frog sartorius muscles had a pronounced effect on the response evoked by exogenous acetylcholine and by indirect stimulation, but had no discernible effect on the response evoked by direct stimulation of the muscle. In Fig. 1 the upper set of records indicate the twitch tension of a directly stimulated muscle in the presence (arrow down) and absence (arrow up) of puromycin. Preceding the application and removal of puromycin no stimuli were delivered for 0.5 min. The lower set of records (Fig. 1) indicate the mechanical response of a sartorius muscle to exogenous acetylcholine (final concentration, 0.055 mM) in the absence of puromycin (extreme left and right records) and 5, 10 and 30 sec after the addition of puromycin.

The mechanical response of indirectly stimulated sartorius muscles decreased in the presence of puromycin (Fig. 2) and the rate of this reduction increased with the frequency of stimulation. The effect of puromycin was reversible (upper records, Fig. 2) at all frequencies of stimulation. A comparable pattern of inhibition of the indirectly evoked contraction was obtained with *d*-tubocurarine at a concentration of 4 μM and the curare effect was also frequency dependent but less so than the puromycin effect (Fig. 3). At very low frequencies of stimulation (1 shock/5 min) the magnitude of the mechanical response to indirect stimulation in the presence of puromycin decreased rapidly during the first 15 min, then more slowly until the response was extinguished after 4–5 hr. In the presence of low concentrations of curare, there was a similar initial rapid decline of the mechanical response followed by stabilization at a low level, and a gradual recovery. The rate and extent of the reduction in twitch tension produced by puromycin was dependent upon the concentration (Fig. 4).

The addition of puromycin to sartorius muscle-nerve preparations in which the end plate potential was monitored after the method of Fatt [12], resulted in a rapid reduction in the magnitude of the end plate potential (Figs. 5 and 6). At the concentrations of puromycin employed (maximum 0.47 mM), the end plate potential was markedly reduced in about 5 min but was not extinguished even after a 60 min sojourn in the puromycin environment. Recovery of the end plate potential after removal of the puromycin containing solutions was never complete (Figs. 5 and 6); this failure to recover was independent of the frequency of stimulation or the time of exposure to puromycin.

The rate of development of the puromycin induced inhibition of the motor end plate potential in driven preparations was independent of frequency in the range tested (1/2 sec to 6/sec), but the extent of inhibition in driven muscle–nerve preparations was lower (mean ratio: $\frac{\text{mv exp.}}{\text{mv cont.}}$ 0.41 ± 0.10 S.E., n = 39) than in non-driven preparations (0.71 ± 0.04 S.E., n = 6) and this effect was also independent of the time of exposure to puromycin.

Acetoxycycloheximide at concentrations from 0.07 0.70 mM had no effect on the magnitude of the motor end plate potential of frog sartorius muscles (23 experiments). Two experiments were performed in which muscle-nerve preparations were exposed to acetoxycyclo-

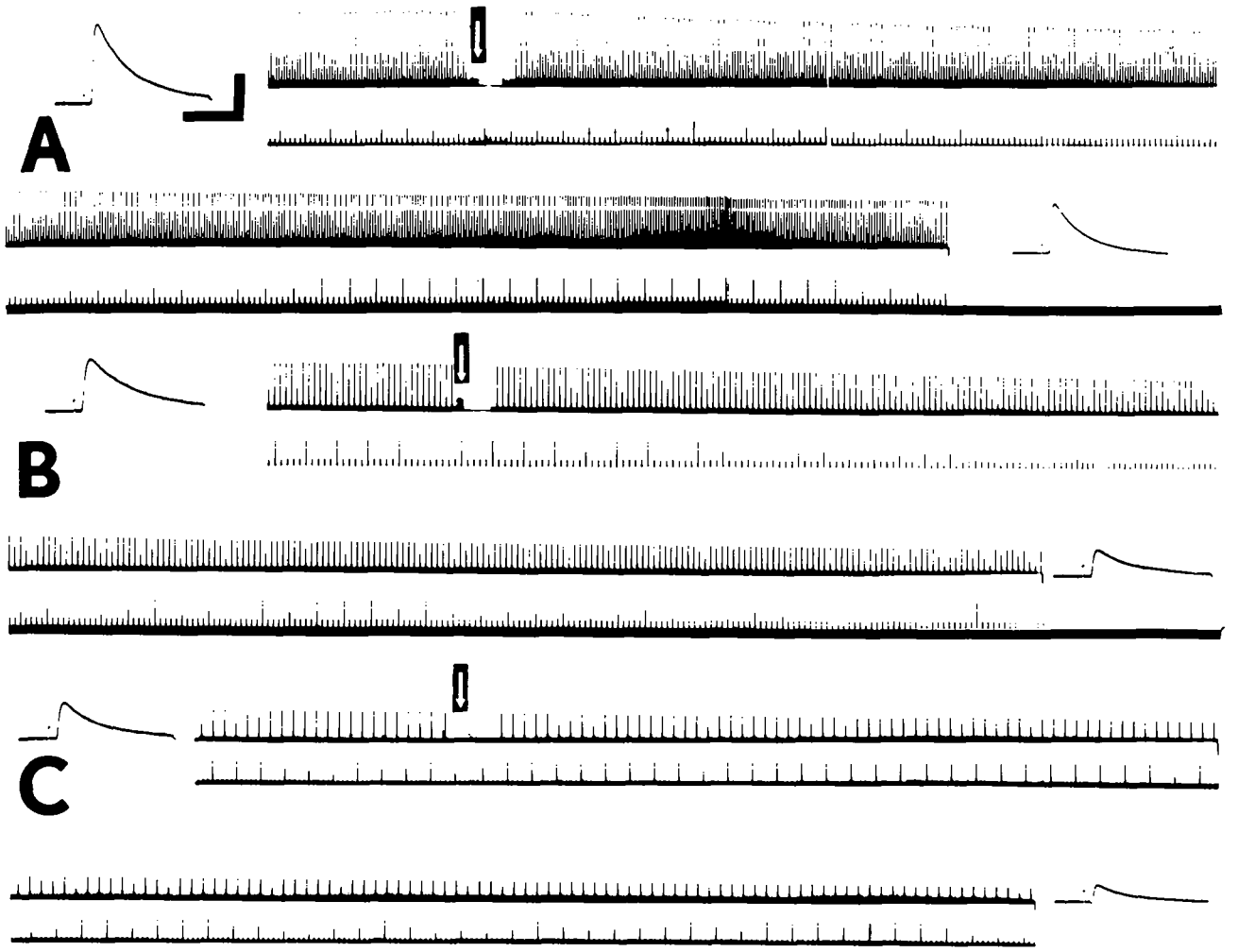

FIG. 5. Records of motor end plate potentials from an indirectly stimulated sartorius muscle obtained with the air-liquid interphase electrode method of Fatt [12]. The liquid level was adjusted for maximal response, about 1 cm below the pelvic end of the muscle. $MgCl_2$ (10 mM) was used to suppress contraction of the muscle. At the time of addition of puromycin (bath concentration, 0.43 mM) (arrow down) stimulation was interrupted for about 10 sec. Single frames were recorded before and after the continuous response trains and one hour elapsed between successive exposures to puromycin. Frequencies of stimulation are: A, 2/sec, B, 1/sec; C, 1/2 sec and calibrations (upper left) indicate 10 msec and 2 mv. The time marks beneath continuous response trains indicate 1 and 5 sec elapsed time.

heximide overnight, also with negative results.

The rate at which puromycin and *d*-tubocurarine produced inhibition both of twitch tension in an indirectly stimulated muscle (Fig. 3) and motor end plate potential (Fig. 6) was similar, although the effective concentration of *d*-tubocurarine was several hundred times lower than that of puromycin. The inhibitory effect of *d*-tubocurarine was more reversible than that of puromycin in all experiments.

The results of the investigations described above indicate that puromycin inhibits neuromuscular transmission by blocking the transitory exictatory effect of acetylcholine on the post synaptic membrane and that this effect mimics that of *d*-tubocurarine, albeit at much lower concentrations. The failure of acetoxycycloheximide to produce any alterations in end plate activity effectively rules out the possibility that puromycin produced its effect by virtue of inhibition of protein biosynthesis.

The results described above indicate that puromycin is not the drug of choice to demonstrate that retention of learned responses is dependent upon protein synthesis and suggest that the disturbance of the retention of learned responses using puromycin must be interpreted with caution.

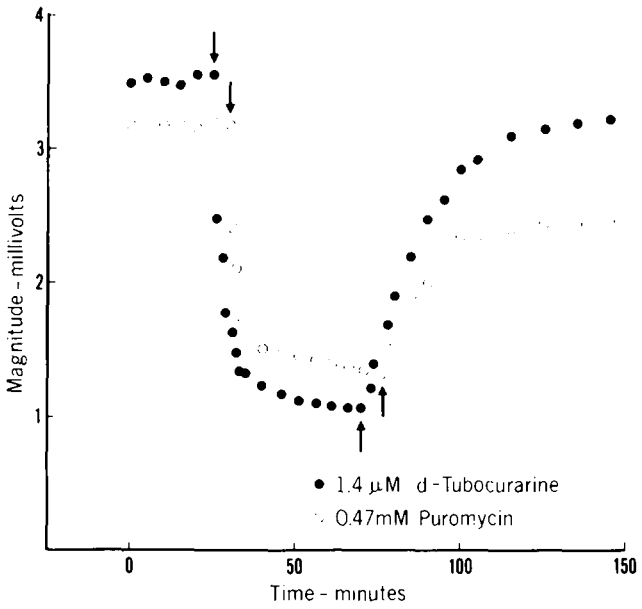

FIG. 6. The magnitude of the motor end plate potential of paired frog sartorius muscle nerve preparations before and after one was exposed to puromycin and the other to *d*-tubocurarine at the indicated concentrations. The downwardly directed arrows indicate the time of application of the drug and the upwardly directed arrows the time of removal of the drug. The stimulation frequency was 1/sec.

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