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Blockade of the Facilitative Action of Neostigmine on Muscular Contraction by the Antibiotic Colistin

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Abstract

In experiments on guinea pig sciatic nerve-tibialis anterior muscle preparations, with the use of suprathreshold stimulation, it was found that neostigmine enhanced the strength of the contraction. If an appropriate interval between doses was used, the facilitative effect of neostigmine was cumulative. There was nearly a fourfold increase in strength of contraction after the third dose of neostigmine. The administration of colistin, an antibiotic possessing some curarelike properties, did not modify the amplitude of the muscular contractions when intravenous doses up to 250 mg/kg were used. If neostigmine was administered not later than 5 min after the injection of colistin, its facilitative effect on the muscular contraction was only slightly diminished; however, if 10 to 20 min elapsed between the administration of the two drugs, the effect of neostigmine was entirely antagonized by colistin. Cumulative doses of neostigmine also failed to have an effect. The nature of the antagonistic effect of colistin and its slow appearance is discussed.

In a prior study (de Naranjo and Naranjo, 1964) it was observed that colistin and succinylcholine produce a mutual potentiation in the neuromuscular blockade. Although the blocking effect of colistin could be due to a direct action of this antibiotic on the motor end plate receptors, the strong potentiation of the duration of the blocking effect of succinylcholine suggested that this response was produced by an inhibition of activity of the true cholinesterase.

Adamson, Marshall, and Long (1960) have already demonstrated that the blockade produced by colistin in sciatic nerve-gastrocnemius muscle preparations in rabbits and chickens was not antagonized by neostigmine. However, the inverse condition, namely the antagonism of colistin on the effect of neostigmine, has not been studied. In addition, when the combined action of two drugs is investigated, it is necessary that the time-courses of their effect coincide at least in part. Neostigmine has a very short latent period, but colistin, on the other hand, produces muscular blockade after a long latency (de Naranjo and Naranjo, 1964).

This investigation was performed to determine whether colistin has anticholinesterase activity.

Materials and Methods

Experiments were performed with male guinea pigs weighing 490 to 520 g. The animals were anesthetized with a mixture of urethane (600 mg/kg) and sodium pentobarbital (10 mg/kg) injected intraperitoneally. The trachea was cannulated for the administration of artificial respiration when necessary, and a polyethylene tube was inserted in the external jugular vein for the injection of drugs. A sciatic nerve-tibialis anterior muscle preparation was set up in the conventional manner, by use of a Brown-Schuster myograph. The tendon

of the tibialis anterior muscle was attached to a flat spring, and the muscle contractions were recorded on smoked paper. Platinum electrodes were placed on the sciatic nerve branch which innervates the tibialis anterior muscle. Square waves of 0.2 msec duration and a frequency of 60 per min, delivered from an electronic stimulator (model 751; American Electronic Labs., Inc.) were used to excite the preparation.

Threshold stimulation, which varied between 0.2 and 0.4 v according to the animal, was used as the standard stimulation. Each experiment was repeated on 5 to 10 animals, and average values and their standard errors were determined.

Colistin methanesulfonate from a batch containing 13,500 units per mg was employed. This antibiotic is a polypeptide ring (Fig. 1) with five molecules of L-diaminobutyric acid and one molecule of each of the following: 6-methyloctanoic acid, L-threonine, and L- and D-leucine. Neostigmine was used as the methylsulfate. Both compounds were diluted in saline solution.

Two series of experiments were carried out. In the first control series, after preliminary assays to standardize the dose of neostigmine, we measured the facilitation of the muscular response to nerve stimulation by administering neostigmine (30 μg/kg) at 10-min intervals. In the second series, colistin (250 mg/kg) was given at various times before starting the series of neostigmine injections.

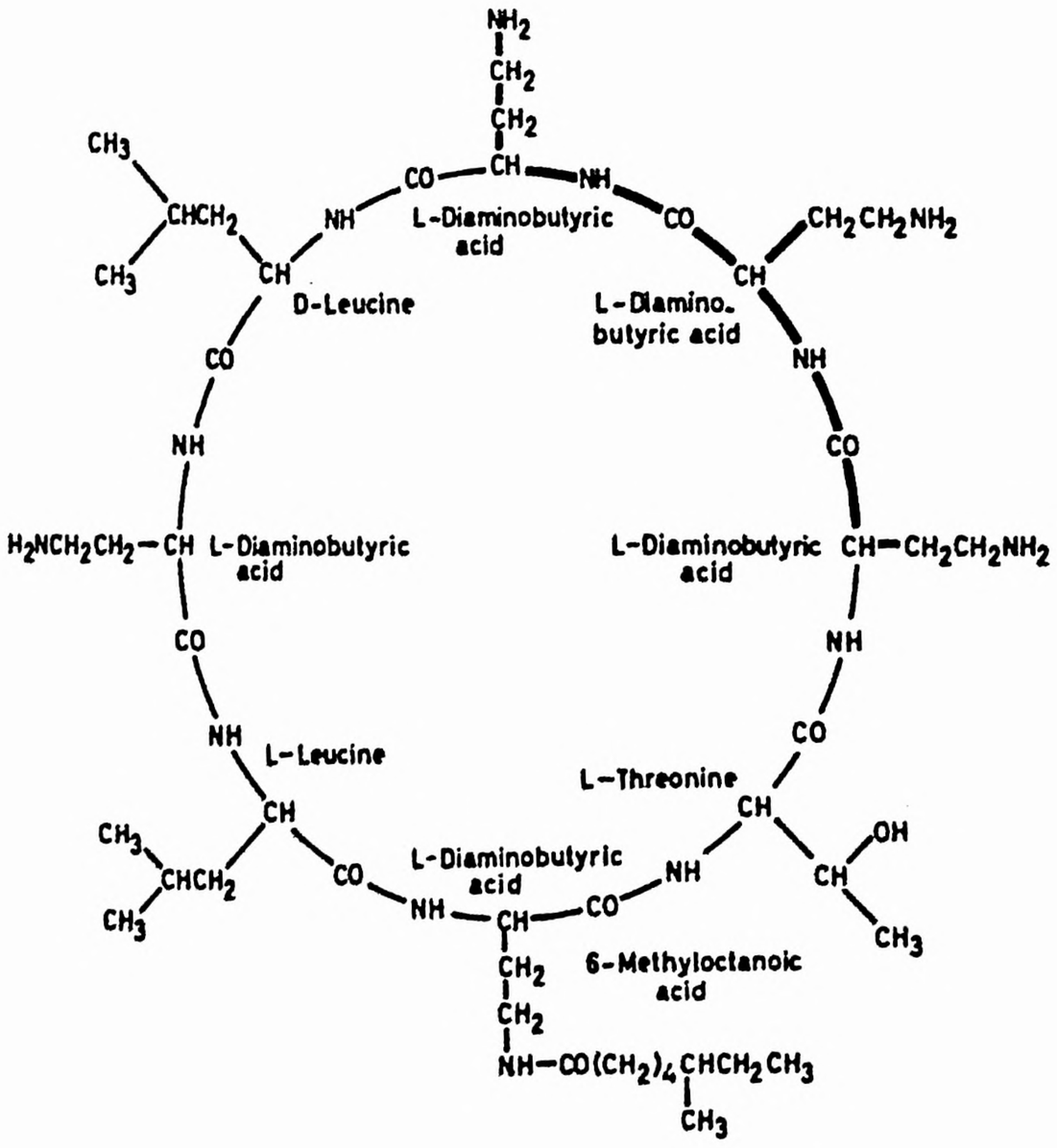

Fig. 1. *Chemical structure of colistin. The thick line indicates that the moiety is probably involved in the anticholinesterase activity of this antibiotic.*

Results

Facilitative effect of neostigmine. The administration of neostigmine produced an increase in the amplitude of muscle contraction. When neostigmine was repeatedly injected, the facilitative effect progressively increased (Fig. 2, Table 1).

Combined effect of colistin and neostigmine. Colistin produced a slight decrease in the amplitude of muscular

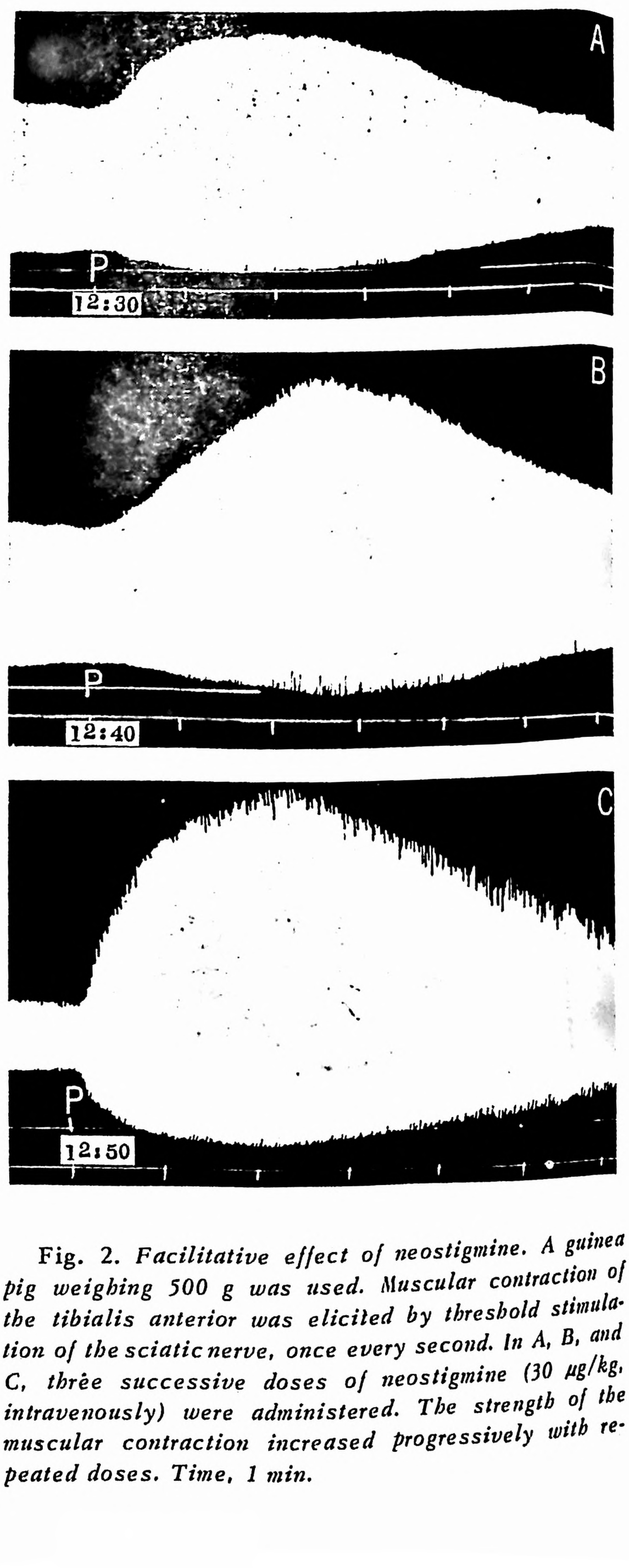

Fig. 2. *Facilitative effect of neostigmine. A guinea pig weighing 500 g was used. Muscular contraction of the tibialis anterior was elicited by threshold stimulation of the sciatic nerve, once every second. In A, B, and C, three successive doses of neostigmine (30 µg/kg, intravenously) were administered. The strength of the muscular contraction increased progressively with repeated doses. Time, 1 min.*

Table 1. *Increase of muscular contraction produced by neostigmine**

No. of doses	Increase in contraction (fold)
1	0.14 ± 0.01
2	1.65 ± 0.18
3	3.85 ± 0.41

*Without neostigmine, contraction was 29.4 ± 4.3 mm.

contraction (Fig. 3) equivalent to 11.5 ± 1.8% of the control contraction. This inhibitory effect began 5 min after the intravenous administration of the drug and reached the peak of its effect after a period of 25 to 30 min.

When neostigmine was administered only 5 min after the injection of colistin, almost no antagonism was observed. When the administration of neostigmine was repeated at 10-min intervals, however, the facilitative effect of neostigmine was reduced (Table 2). If the first dose of neostigmine was administered 30 min after colistin, a weak antagonism was still observed. However, with the third dose of neostigmine (50 min after colistin) there was no facilitative effect, and complete antagonism was observed. After colistin had been administered for 2 hr, its antagonistic effect against neostigmine began to disappear and was over after 3 hr (Fig. 4).

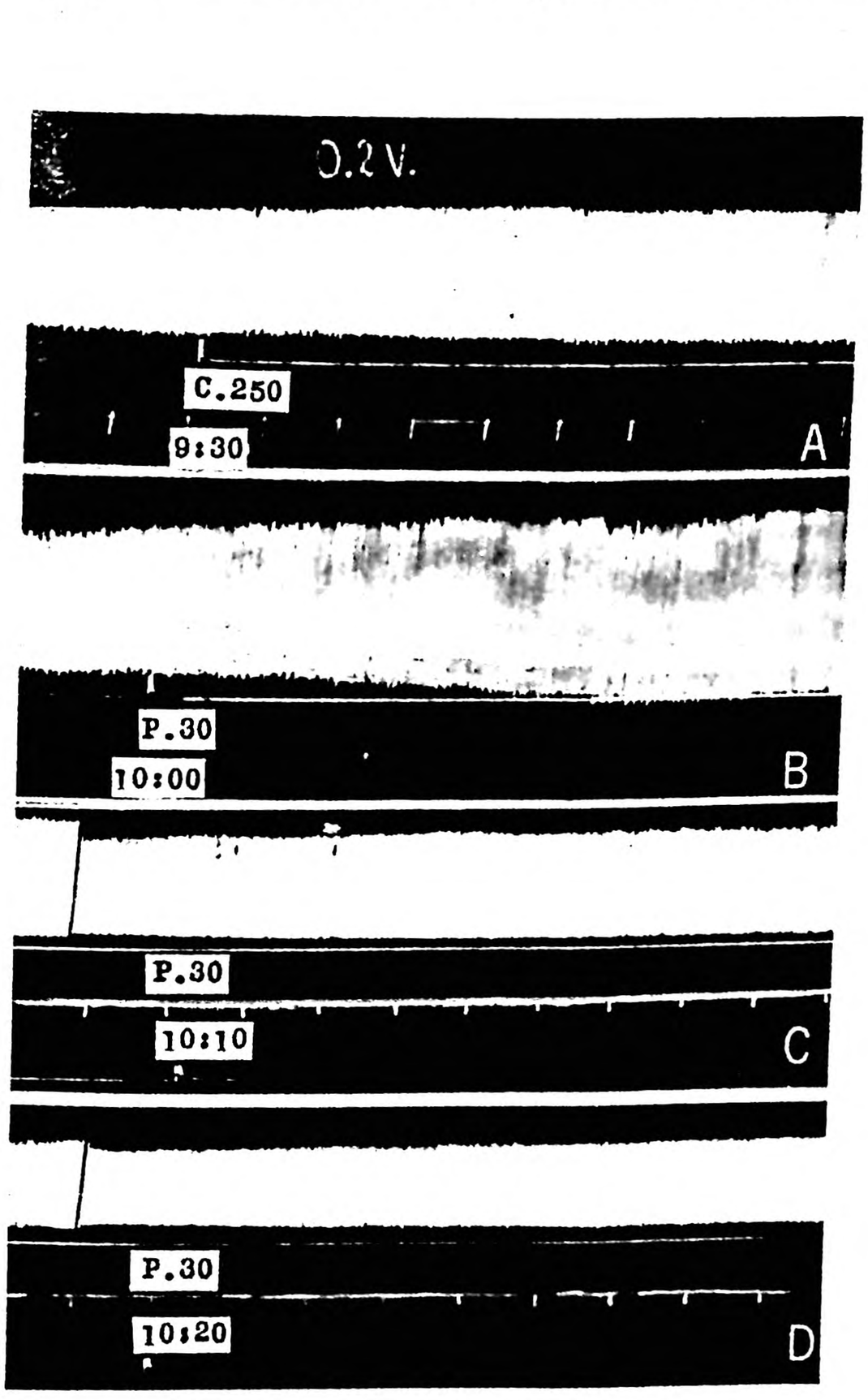

Fig. 3. *Inhibition of the facilitative effect of neostigmine by colistin. A guinea pig weighing 490 g was used. Muscular contraction of the tibialis anterior was elicited by threshold stimulation of the sciatic nerve, once every second. (A) Intravenous injection of colistin (250 mg/kg). (B, C, D) Three successive intravenous doses of neostigmine (30 μg/kg). The facilitative effect of the muscular contraction produced by neostigmine was completely prevented by colistin.*

Discussion

The results demonstrate that colistin, in high dosage, can entirely antagonize the facilitative effect of neostigmine on the contraction of striated muscle, and that it can block the cumulative effects of neostigmine. It is well known that, although neostigmine can directly stimulate the cholinergic receptors of the motor end plate, its principal action is to inhibit cholinesterase, which in turn permits a larger number of acetylcholine molecules to act on the receptors for a longer period.

At first glance it may be assumed that if a certain drug, e.g., colistin, blocks cholinesterase it would sum its effects with those of neostigmine. The

Table 2. *Antagonism of colistin on the facilitative effect of neostigmine*

Series	No. of doses	Interval between drugs*	Increase of the muscular contraction (fold)
		min	
I	1	5	0.13 ± 0.01
	2	15	1.21 ± 0.13
	3	25	2.48 ± 0.28
II	1	30	0.11 ± 0.01
	2	40	0.05 ± 0.01
	3	50	0

*Neostigmine was administered at the indicated intervals after the administration of colistin.

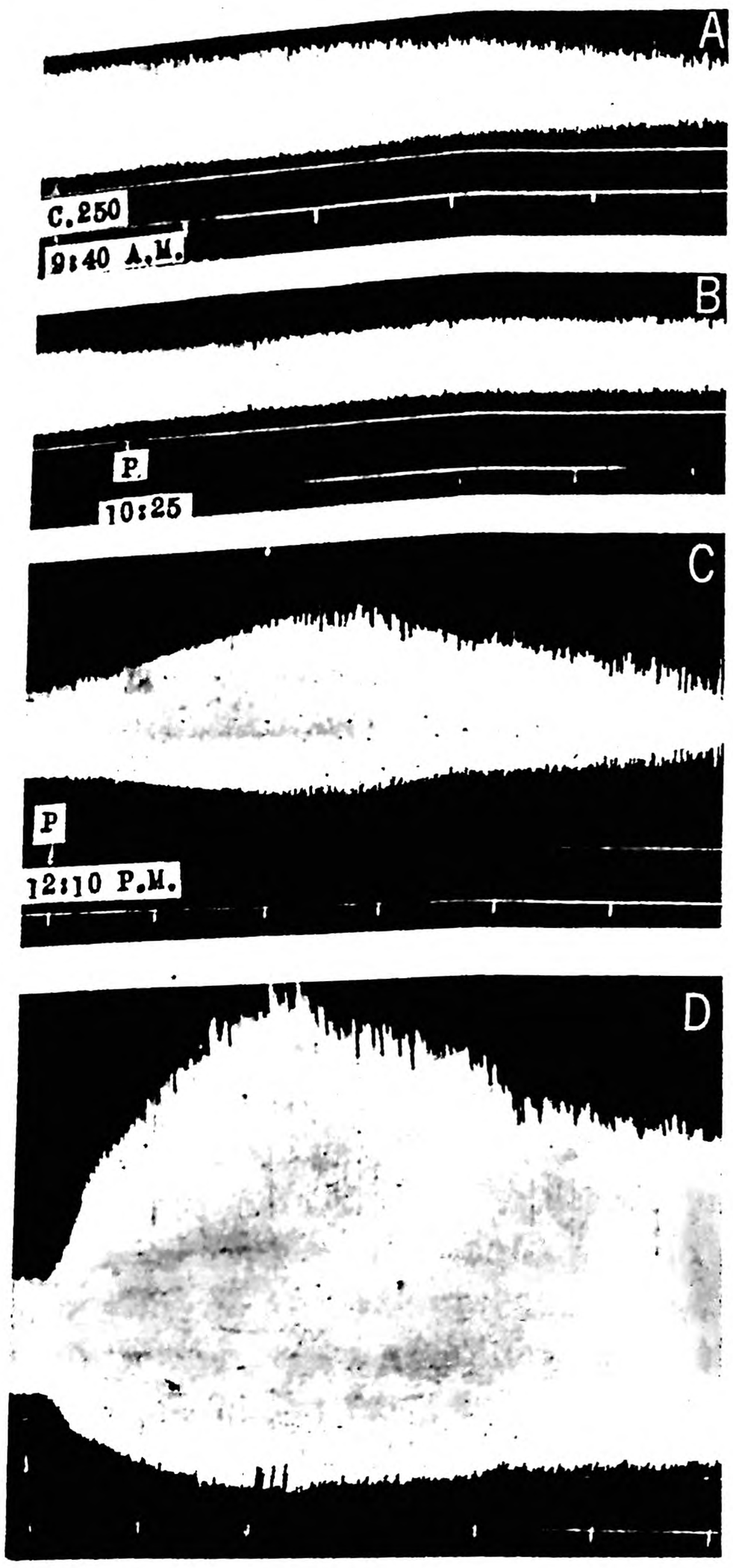

Fig. 4. *Recuperation of the facilitative effect of neostinmine. A guinea pig weighing 480 g was used. (Details of the preparation as in Fig. 3). (A) Intravenous injection of colistin (250 mg/kg). (E, C, D) Three successive doses of neostigmine (30 μg/kg) given 45(B), 150(C), and 170(D) min after colistin.*

present data show, on the contrary, than antagonism is produced.

The particular design of the present work leaves only a narrow margin for interpretation and hypothesis. From a previous investigation (de Naranjo and Naranjo, 1964) and other papers. (Adamson et al., 1960; Schwartz et al., 1960), as well as from the present experiments, we concluded that colistin: (i) acts directly on the striated muscle (since it is capable of inhibiting the muscular contraction elicited by direct stimulation of the fiber); (ii) acts also at the level of the neuromuscular junction, partly blocking the motor end plate receptors; and (iii) blocks cholinesterase activity.

The conjugation of colistin with cholinesterase was slow, reaching its maximum, under the conditions of the present experiments, after 25 to 60 min. This reaction appears to be irreversible or only slowly reversible. This might explain why the facilitative action of neostigmine was not antagonized when it was given only a few minutes after the colistin. Neostigmine conjugates with cholinesterase more quickly and more selectively than does colistin. On the other hand, when enough time has elapsed, the antagonism of the two drugs may be complete and long lasting. If colistin acts only by blocking cholinesterase, without avoiding the action of acetylcholine on the motor end plate receptors, it should produce a facilitative effect on the muscular contraction similar to that produced by neostigmine.

The slow and delayed anticholinesterase activity of colistin was also demonstrated in a previous communication (de Naranjo and Naranjo, 1964), in which the potentiation of the neuromuscular blockade by succinylcholine was described. This effect also appeared after 20 min or more of latency.

The succinylcholine effect increased not only in intensity but particularly in duration. In fact, the duration of the blockade was increased 15.3 times, a phenomenon that necessarily must be due to the long-lasting blocking effect of colistin on the cholinesterase activity.

On basis of the investigations carried out by McCarthy and Chenoweth (1962), in which they demonstrated that a series of polyglycol diamine compounds produced curarelike effects, it is possible to attribute the anticholinesterase activity of colistin to one or both kinds of the following molecular moieties: $NH_2(H_2)^2$, $CH\text{-}NH\text{-}CO\text{-}CH(CH_2)_2NH_2$ and $(CH_3)_2CH\text{-}CH_2\text{-}CH\text{-}NH\text{-}CO\text{-}CH\text{-}(CH_2)_2NH$.

Polymyxin E is considered to be the same substance as colistin (Wilkinson, 1963), and Cohen, Purdy, and Kushnick (1954) have shown that polymyxin B, an

antibiotic closely related to colistin, is able to inhibit the esterase activity of several microorganisms. Moreover, Mohan and Pianotti (Bacteriol. Proc., p. 99, 1960) observed that colistin at a certain dose level inhibited the oxidative metabolism of 2-keto-gluconate, acetate, and oxalate but not of glucose, and Mohan et al. (1962) concluded that the most sensitive site of action of this antibiotic in *Pseudomonas aeruginosa* is at the acetate level.

Our findings confirm the observation that colistin has a long-lasting anti-esterase activity, and suggest that the mechanism of antibiotic action may be ascribed at least in part to it. Another possible mechanism which has been described by Mohan and Pianotti (Bacteriol. Proc., p. 99, 1960) and others (Newton, 1953; Few, 1954; Valentini et al., 1958) suggests some alteration of the membrane permeability and leakage of cytoplasmic materials such as inorganic phosphate, sugars, and proteins.

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