THE ACTION OF SOME ANTIBIOTICS ON THE HUMAN INTERCOSTAL NERVE-MUSCLE COMPLEX

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SINCE Pridgen reported four cases of respiratory paralysis following the intraperitoneal administration of neomycin,[1] six more cases of a similar complication have been published.[2-7] The gravity of this complication is evident from the high mortality (40 per cent) attending the above series. It appears that the combination of ether and neomycin was the prime factor in the production of respiratory paralysis.[8] Immediate, vigorous and prolonged artificial ventilation seems to have played a more important part in resuscitating the survivors than any antagonist administered.

Experimental animals die asphyxial deaths when lethal doses of certain antibiotics are administered to them parenterally. Brownlee and Bushby, in their study of polymyxin,[9] described such deaths and extended their work to include studies on the isolated rat diaphragm. These studies showed that polymyxin in high doses had a neuromuscular blocking effect. Pharmacologic studies on intact animals by Brazil and Corrado[10] and by Pittinger and Long[8] have indicated that streptomycin and neomycin in toxic doses possess similar paralyzing properties.

The work to be presented here is an extension of the above studies. The test material used is the human intercostal muscle preparation and a new technique has been used in those experiments designed to show synergism between ether and neomycin. The antibiotics studied were bacitracin, polymyxin B, streptomycin, neomycin and kanamycin.

METHODS

Strips of human intercostal muscle were removed during thoracotomy for surgical diseases of the chest. Further dissection was performed in the laboratory and the final preparation was suspended on a plastic holder, and immersed in modified Krebs saline as described previously.[11] The saline was continuously gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide.

Once every 10 seconds the muscle was stimulated. These stimuli were applied alternately, first to the muscle directly and second indirectly through the nerve fibers lying within the muscle tissue. The direct stimuli were obtained from a clipped condenser discharge stimulator.[11] The indirect pulse of this stimulator (with some modifications in the circuit) was used to trigger a Dumont 404 R pulse generator, the output of which was used as the indirect stimulus.

Our inability to maintain constant concentrations of volatile anesthetics over long periods of time in previous experiments,[12] was largely overcome by using the closed chamber described by Whalen.[13] The chamber used in the experiments was larger (48 ml. total internal capacity to Whalen's 13 ml.) and was equipped with two more platinum electrodes, placed at each end of the muscle bundle. These electrodes were used for direct stimulation of the muscle. Whalen's mass electrodes were coated with plastic, leaving a bare area of platinum in their middle one-third. These electrodes were used to stimulate the muscle indirectly through its nerve fibers. The drain was sealed with a thick rubber cap, through which a 12 inch, 27 gauge B-D needle was pushed up to the pin valve. This needle served to introduce drugs into the chamber without opening it to the atmosphere. Liquid ether was injected in microliter quantities with a Gilmont Micropipet-Buret. A diagram of the chamber is shown in figure 1. For details of the use and performance of these chambers reference should be made to Whalen's article.[13] Details of the experiments performed in the present study are included in the results.

Methods Employed in Calculating Percentage Blocks. During the progression of a prolonged experiment, a number of factors have

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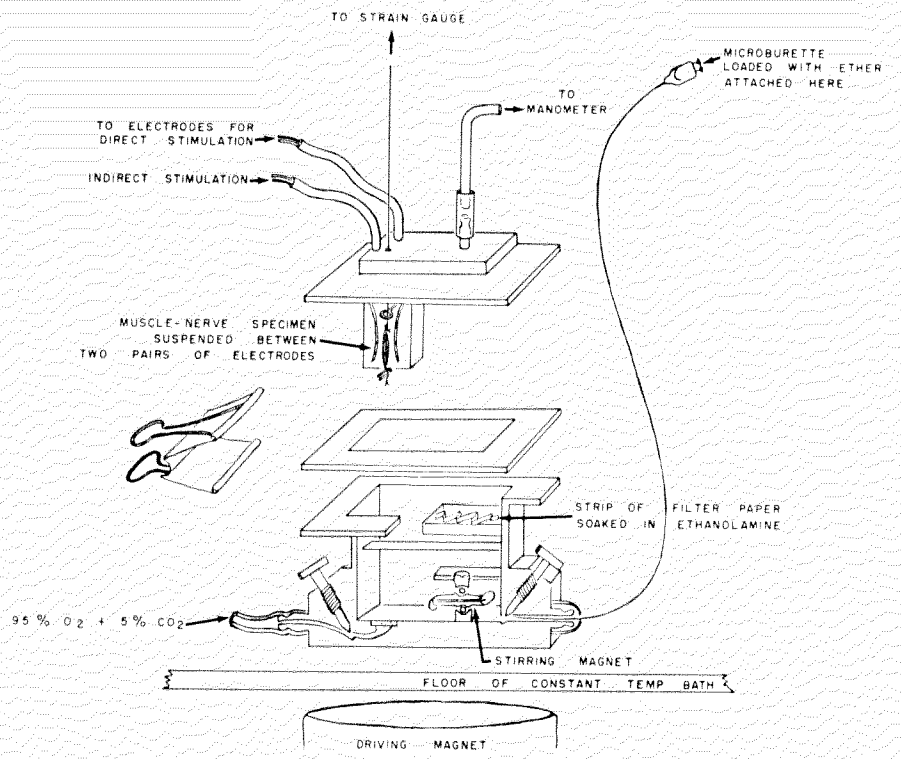

FIG. 1. Exploded diagram of closed chamber used to study the effect of known, constant concentrations of ether over long periods of time. The anterior wall of the chamber is cut away to show details of the interior.

to be considered when calculating the difference in height of the contractions evoked by direct and indirect stimulation at any given time. Fortunately, these factors are constant from experiment to experiment and therefore can be easily allowed for during any single experiment.

DECREMENT: During the many hours necessary for the successful completion of some of these experiments, the strength of contraction diminishes progressively, almost imperceptibly at first but then more rapidly during the later stages. The reason for this is probably the artificial medium in which the muscle performs. Obviously then, one hundred per cent of twitch height at the end of a long experiment is a fraction of that at the beginning of the experiment.

"APPARENT" BLOCK: During the control period, most specimens show some difference in the height of contractions produced by direct and indirect stimulation. Contractions evoked by indirect stimulation are always smaller than those produced by directly stimulating the muscle itself. This difference in height varies from 1 or 2 mm. in good specimens to much larger amounts in muscles presumably damaged during dissection. (Myasthenic muscle for some unknown reason invariably shows a large apparent block.) Since this difference in contraction height is a measure of neuromuscular block, and since it is present before any drug is added to the bathing medium, it has been named the "apparent" block. Fortunately, this difference in height remains constant throughout the experiment (in the absence of fatigue) and can be accounted for at any particular time by deducting it from the height of the direct response.

Fatigue is not significant, as the slow rate of stimulation (6/minute) does not produce fatigue however long the experiment. At the higher rates of stimulation employed by some investigators, fatigue would itself be an im-

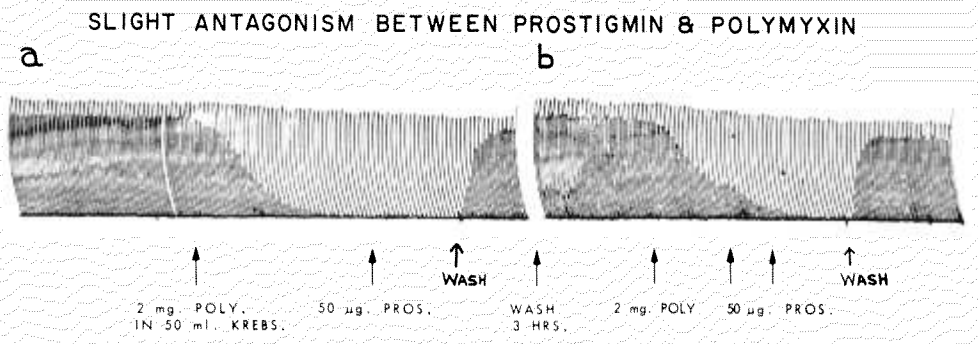

FIG. 2. Neuromuscular blocking action of polymyxin B, (2a) Antagonistic action of prostigmin (Pros.) added to bathing fluid after indirect response is completely suppressed by polymyxin. (b) Prostigmin (Pros.) added while height of indirect response is still falling.

portant factor in the production of a neuromuscular block and would have to be allowed for by a consideration of the increase in the "apparent" block at the conclusion of the experiment.

RESULTS

Bacitracin had such a slight action that it is not reported in this study, and kanamycin did not appear to affect the neuromuscular preparation at all.

Polymyxin B (6 Experiments). Weight for weight polymyxin had the most powerful neuromuscular blocking activity of the antibiotics tested. In the experiment recorded in figure 2, the effect of polymyxin B sulfate in a concentration of 40 µg./ml. Krebs saline showed a rapid block to indirect stimulation. To study the effect of prostigmin on this block, two experiments were performed. In one (fig. 2a), prostigmin was added after the indirect response was completely suppressed by the

NEUROMUSCULAR BLOCK IN HUMAN INTERCOSTAL MUSCLE PRODUCED BY STREPTOMYCIN

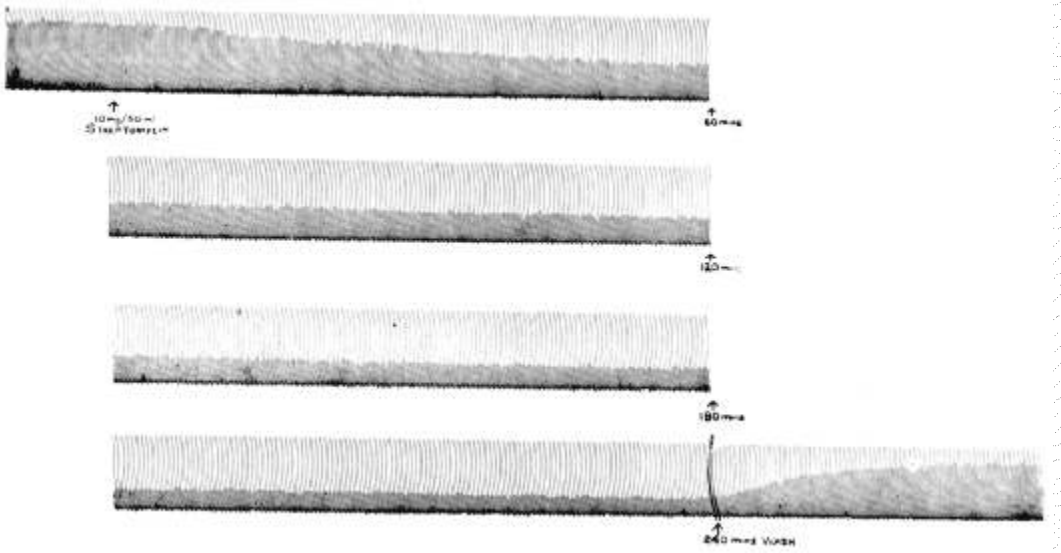

FIG. 3. Effect of adding 10 mg. streptomycin to 50 ml. bathing fluid. Result is a steady 69 per cent block from 3 hours onwards.

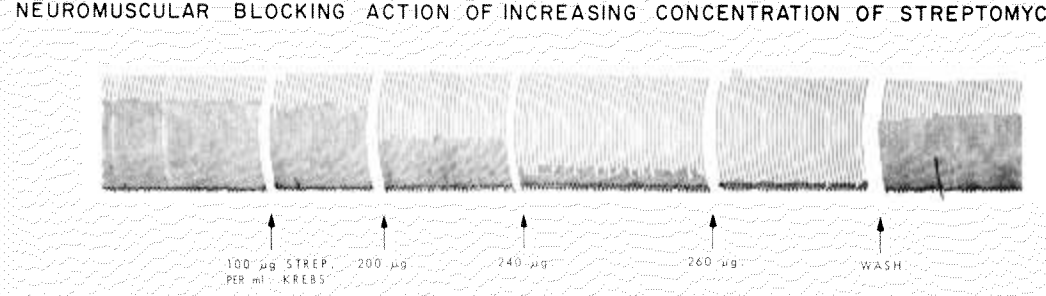

FIG. 4. Effect of increasing concentration of streptomycin in bathing saline. The percentage block is seen to increase as the concentration of streptomycin is increased. Each break in the record represents a pause of 2 hours so that a state of equilibrium has time to be established. Drug concentration increased as shown.

antibiotic. In the second (fig. 2b), prostigmin was added while the height of the indirect response was still being reduced.

Prostigmin appears to exert a weak antagonism to the neuromuscular blocking action of polymyxin, as is evidenced by the slight reappearance of the indirect response in figure 2a, and by the two small waves which slow down the fall of the indirect response in figure 2b. Theoretically, if the concentration of polymyxin were lower, and the prostigmin had been added after the blocking effect of polymyxin had reached a state of equilibrium, then the antagonism between these two drugs would have been more obvious. Unfortunately, we have been unable to demonstrate a marked antagonism whatever the circumstances of the experiment, while Brownlee and Bushby failed to find any evidence of antagonism in their experiments.[9]

Streptomycin (6 Experiments). When 10 mg. of streptomycin were added to 50 ml. of Krebs saline, the resulting effect on the neuromuscular preparation was as shown in figure 3. The progress of the block was rapid during the first hour (54 per cent), and then it slowed down until a constant level at about 3 hours was reached (60 per cent at 2 hours, 63 per cent at 2½ hours, 69 per cent at 3 hours, 69 per cent at 3½ hours). The establishment of a steady state of paralysis [14] and the fact that the direct response was not affected suggest that this block is truly curariform. The next experiment as represented in figure 4 showed an increasing percentage block resulting from an increase in the dose of streptomycin. Since these determinations were made at or near equilibrium, they were not complicated by diffusion kinetics, and hence are true parameters of this particular action of the drug.[15]

Figure 5 shows the antagonistic action of prostigmin to a partial, steady 50 per cent block produced by the addition of 10 mg. of streptomycin to the bathing saline. On adding prostigmin, the block was soon reduced to 20 per cent, while on washing the preparation with fresh Krebs saline, the block was eliminated completely.

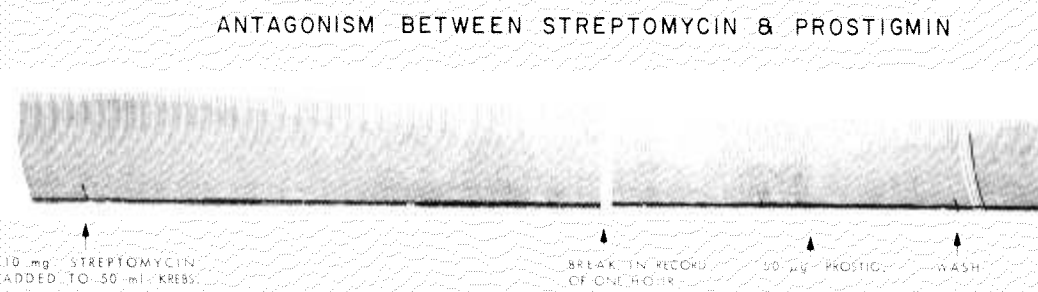

FIG. 5. Demonstration of antagonistic effect of prostigmin to the neuromuscular blocking action of streptomycin.

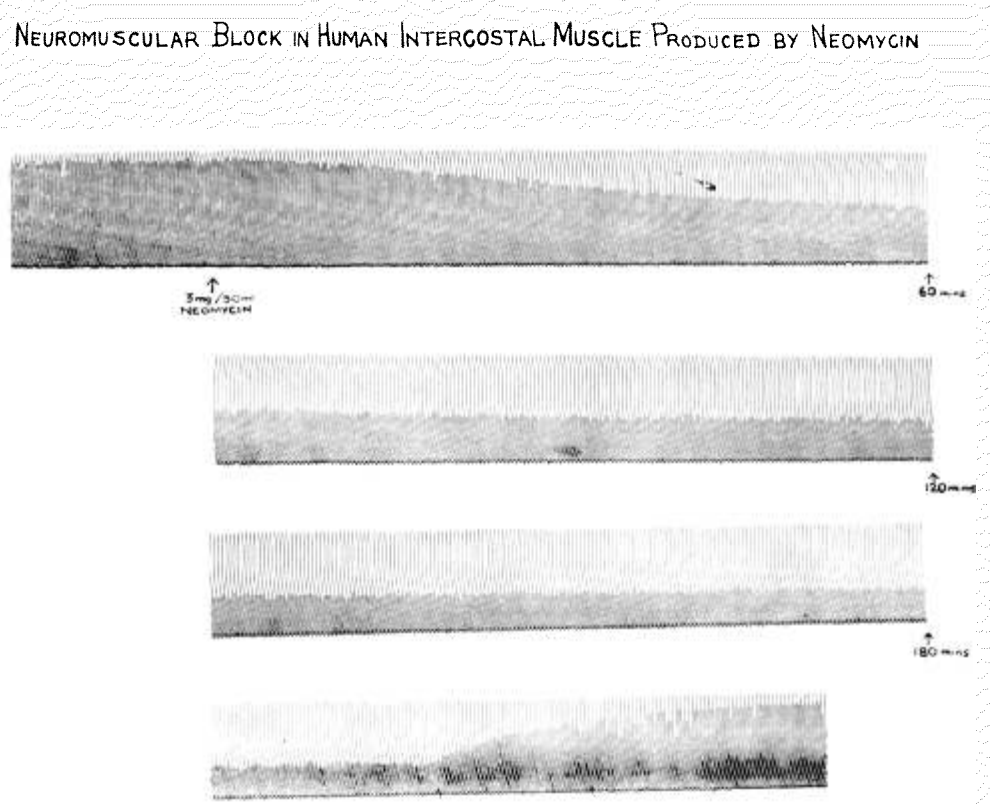

FIG. 6. Constant, steady 60 per cent block produced by the addition of 3 mg. neomycin to 50 ml. Krebs saline.

Neomycin (*25 Experiments*). The addition of 3 mg. of neomycin sulfate to 50 ml. of bathing saline produced a neuromuscular block which progressed at a rate comparable to that of a block produced by 10 mg. of streptomycin. In figure 6 the progression of the block was as follows: 44 per cent at 60 minutes, 54 per cent at 90 minutes, 57.5 per cent at 120 minutes, 60 per cent at 150 minutes, 60 per cent at 180 minutes. Figures 7 and 8 represent records from experiments which demonstrated the establishing of steady states of partial paralysis. These were induced first by gradually increasing the concentration of neomycin in the bath-

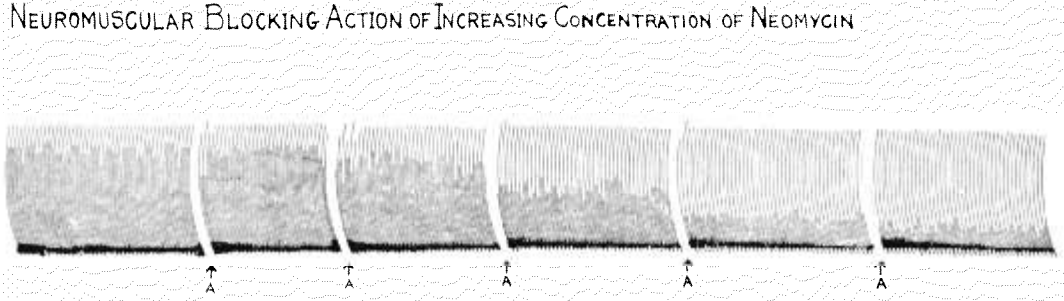

FIG. 7. Increasing steady partial blocks produced by increasing the concentration of neomycin in bathing fluid. At each point marked A, the concentration of neomycin has been increased by 20 μg./ml. of bathing fluid. There is a break of 2 hours between each section of record.

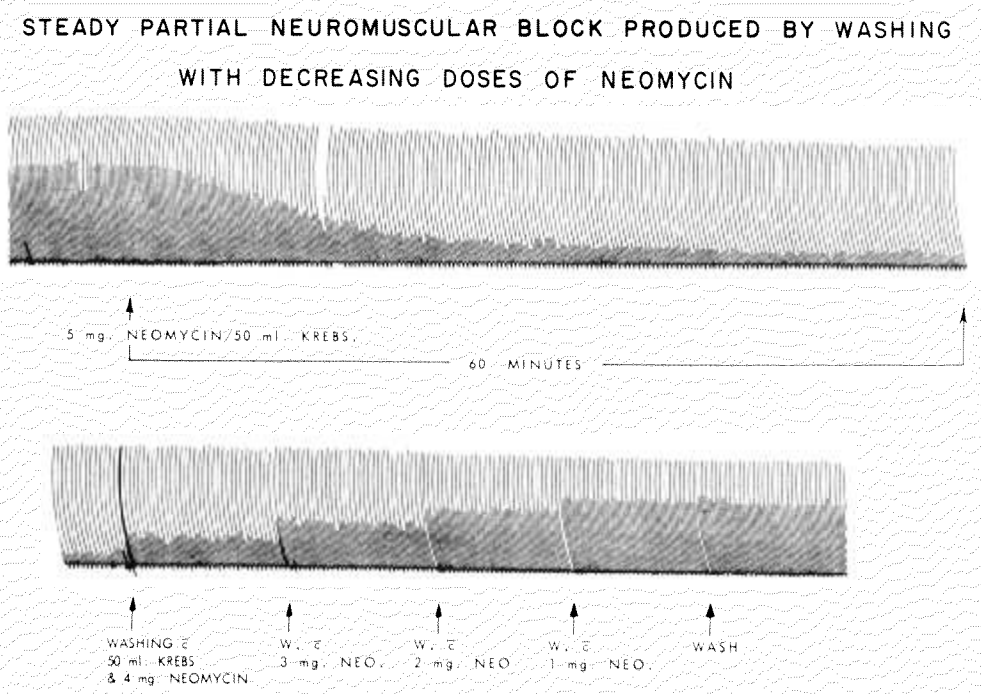

FIG. 8. Decreasing steady partial blocks produced by decreasing concentration of neomycin in bathing fluid. Amount of drug reduced as shown, followed by a pause of 2 hours before recording again. See text for a comparison of partial blocks in this experiment and those recorded in figure 7.

ing fluid and second by gradually decreasing it. A comparison between the percentage blocks produced by the same concentration of neomycin in these two experiments shows that the dose-response relationship is similar regardless of whether the drug is diffusing towards or away from the receptor site (table 1).

Figure 9 shows the antagonistic effect of prostigmin in a concentration of 0.5 microgram per ml. saline to a steady 72 per cent block produced by the addition of neomycin (100 μg./ml. saline) two hours previously. After the addition of prostigmin, the block was reduced to 44 per cent.

TABLE 1

COMPARISON BETWEEN PERCENTAGE BLOCKS (AVERAGE OF 6 EXPERIMENTS) PRODUCED BY NEOMYCIN DIFFUSING TOWARDS AND AWAY FROM RECEPTOR SITE (DISCUSSION IN TEXT)

Concentration of Drug in μg./ml. Saline	Percentage Blocks: Drug Diffusing into Muscle	Percentage Blocks: Drug Diffusing Out of Muscle
20	3	0
40	12	10
60	41	38
80	72	59
100	84	83

Figures 10 and 11 are representative records of experiments designed to illustrate the potentiation of the neuromuscular blocking action of neomycin by the presence of known amounts of ether vapor in the chamber. The apparatus used is illustrated in figure 1. The experiments were performed as follows: The nerve-muscle preparation was suspended between the electrodes by monofilament nylon threads. The upper thread was passed through a mercury seal in the cover. Twenty-five milliliters of Krebs saline in the lower part of the chamber were equilibrated by continuous gassing with 95 per cent oxygen and 5 per cent carbon dioxide. A strip of filter paper soaked in diethanolamine was put in a small, shallow tray, which was then introduced into the lower chamber well above the level of the Krebs saline. The performance of diethanolamine as a carbon dioxide absorber is adequately de-

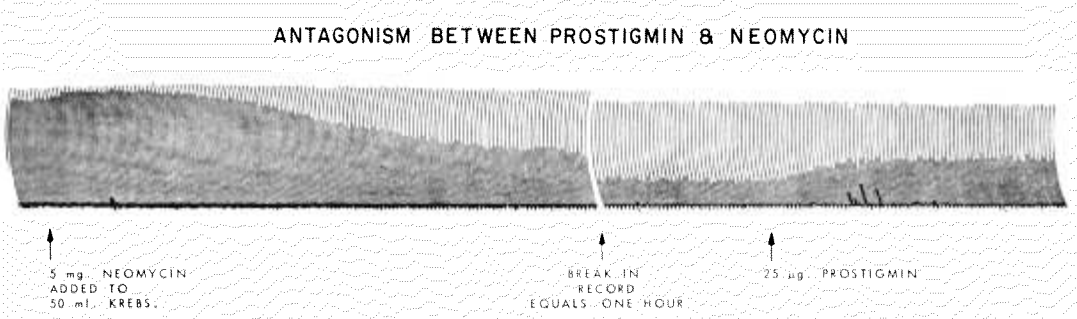

FIG. 9. Demonstration of the antagonistic effect of prostigmin to the neuromuscular blocking action of neomycin.

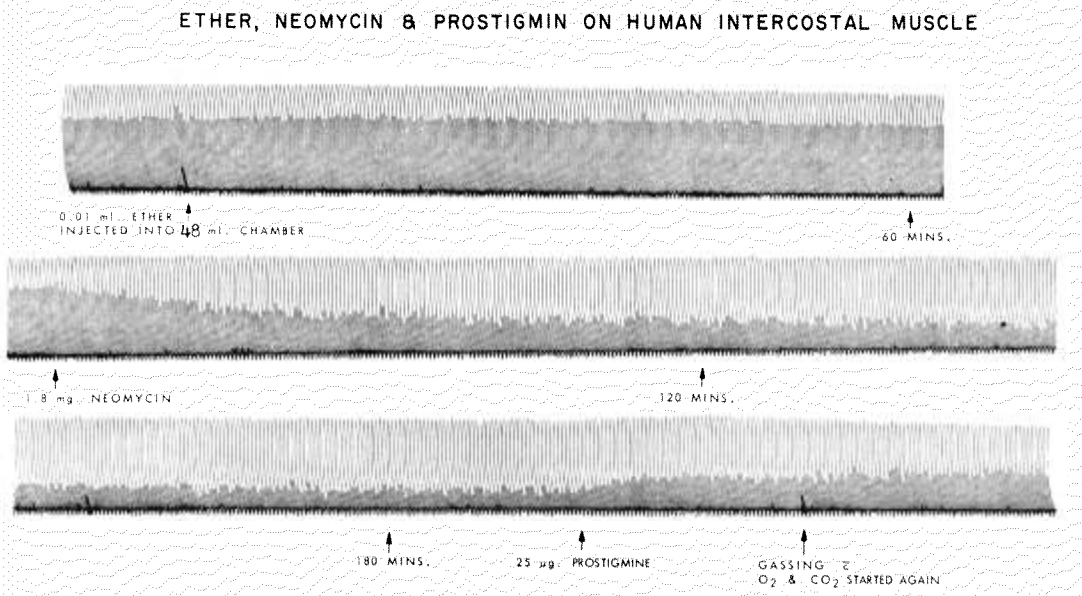

FIG. 10. Increased neuromuscular blocking activity of neomycin in the presence of a small amount of ether vapor which has no neuromuscular blocking action before the addition of neomycin.

scribed by Whalen.[13] The lid (bearing the muscle) was then lowered onto the chamber and sealed with a thin layer of silicone grease on each side of a gasket and two or three paper clips. The chamber was then completely submerged in a bath containing water at 37 C. The glass tube leading to the manometer was then attached. Gassing was stopped by closing the pin valve, but adequate oxygenation of the preparation was assured by constantly stirring the fluid medium with the magnets. Stimulation of the muscle and the recording of its contractions were as usual. Ether was introduced into the chamber with a microburet, the pin valve proximal to the needle was then closed and the microburet was removed. As soon as it was injected, the liquid ether was volatilized, as it was now above its boiling point. This expansion was transmitted to the manometer. The barometric pressure in the chamber was then restored to atmospheric by dropping the columns of mercury in the manometer until both columns in the U tube were at the same level. The muscle was then allowed to contract in the presence of ether for over one hour so that equilibrium would be established between the amount of ether vapor above the level of the Krebs saline and the amount in solution. The concentration of ether vapor in the chamber

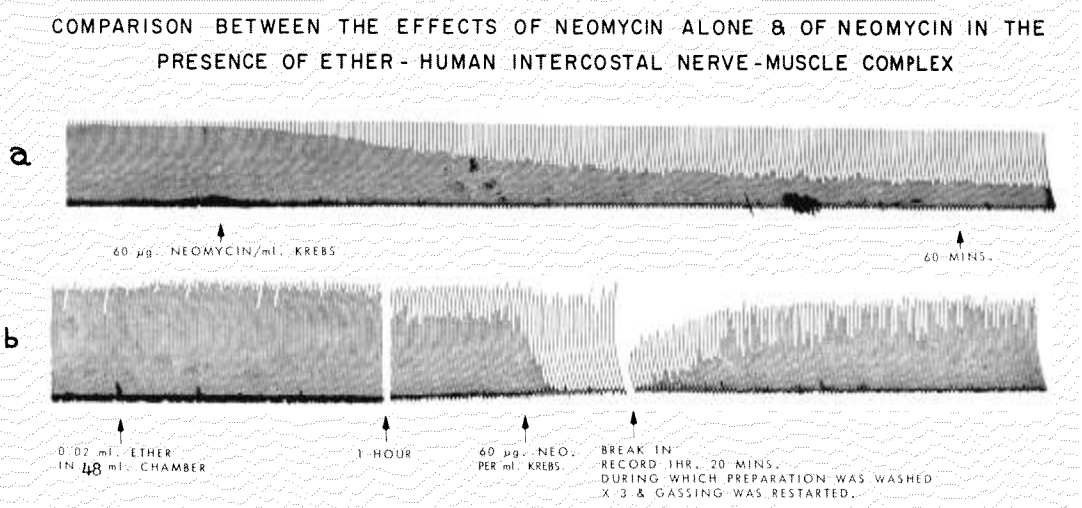

FIG. 11. Two experiments performed at the same time on muscle specimens removed from the same patient. (a) Usual open bath experiment to demonstrate the blocking action of 60 μg. neomycin per milliliter Krebs saline. (b) Closed bath experimeat showing marked potentiation of blocking action produced by the same concentration of neomycin as in (a) by the presence of ether in constant concentration.

was calculated from the movements of the mercury columns in the manometer and from Charles' law.[16] After equilibrium was assumed to be established, any neuromuscular block resulting from the ether was calculated, and neomycin was injected through the 12 inch needle after opening the pin valve. After the injection of any drug, the needle was flushed with 0.2 ml. of saline withdrawn from the chamber with a tuberculin syringe on a three-way stopcock attached to the hub of the 12 inch needle.

In the presence of 6.25 per cent ether (fig. 10) no neuromuscular block was produced by the ether itself, but neomycin, in a concentration of 72 μg./ml. Krebs, induced a steady 65 per cent block. This was reduced to a 44 per cent block by the introduction of prostigmin (1 μg./ml. Krebs). Towards the end of the experiment, gassing was begun again. The ether was blown away resulting in a further reduction of the block to 28 per cent within 10 minutes. The conclusions drawn from this experiment are: that neomycin produces a far greater block in the presence of ether than in its absence (*cf.* figs. 7 and 8); that prostigmin counteracts the block produced by neomycin but not that produced by ether, and that a concentration of ether insufficient to produce a neuromuscular block by itself does produce a certain degree of block in the presence of neomycin. These conclusions appear to indicate that there is a true potentiation between ether and neomycin.

Figure 11 shows records from two different experiments performed at the same time on two specimens of intercostal muscle removed from the same patient. Figure 11a illustrates the usual open bath experiment showing the neuromuscular blocking action of neomycin in a concentration of 60 microgram per milliliter Krebs. Figure 11b depicts a closed-bath experiment showing the neuromuscular blocking effect of the same concentration of neomycin in the presence of 12.5 per cent ether vapor. This high concentration of ether itself produces a 28 per cent block at the end of one hour. On the addition of neomycin, the sudden and complete disappearance of the response to indirect stimulation was a dramatic demonstration of the potentiation between ether and neomycin at high concentrations.

DISCUSSION

There appears to be no doubt that the dose of antibiotic necessary to produce an appre-

ciable neuromuscular block is far above its therapeutic range. To quote the observations of Brownlee and Bushby [9] concerning the acute toxicity of polymyxin B to mice, "Toxic doses caused immediate symptoms consisting of vasoconstriction, muscular incoordination and respiratory distress. At this stage stimulation of the central nervous system was shown by occasional strychnine-like convulsions. Complete flaccidity of skeletal muscles followed, with dyspnoea and apnoea. Arrest of the heart and death from anoxia followed in three or four minutes. In animals which recovered from near-lethal doses, the curare-like effect on muscle was more in evidence and gave way to a phase of vasodilation and ultimate recovery."

Since polymyxin and streptomycin have not yet been implicated in inducing a paralytic state in clinical practice, the rest of the discussion will be restricted to the use of neomycin. In clinical practice a great deal of attention has been paid to such chronic toxic effects as damage to the kidney and to the eighth cranial nerve, and to such acute toxic effects as anaphylactoid shock.[17] Paralysis resulting from the parenteral administration of certain antibiotics, however, remained restricted to laboratory observations until recent years when it has become common practice to introduce large amounts of a potentially toxic agent into the peritoneal cavity, considerable amounts of which are absorbed rapidly into the circulation.[18] Recent reports indicate that man can tolerate 1.0 Gm. of neomycin per day parenterally for one week without harm.[19, 20] It is significant therefore, that the cases of prolonged respiratory paralysis reported in the literature have all been observed in patients under anesthesia. The studies of Pittinger and Long,[8] and the work presented here indicate that there is a synergism between ether and neomycin. In all these cases it is obvious that it is the combination of these two agents that has been the precipitating factor rather than an overwhelming dose of either drug by itself. In a patient receiving, or scheduled to receive parenteral neomycin, the anesthetic agent of choice would appear to be cyclopropane because of its positive inotropic action.[21] Relaxation to the surgeons' requirements would have to be achieved by some regional procedure.

Summary

The increasing use of parenteral neomycin has presented the anesthesiologist with a complication previously unheard of in clinical practice, prolonged respiratory paralysis due to an antibiotic. It is argued that it is not the antibiotic by itself that produces the paralysis, but the combination of the antibiotic with ether which possesses muscle-paralysing properties of its own. Experiments were performed to demonstrate the large doses of polymyxin, streptomycin, and neomycin required to produce paralysis and to demonstrate the potentiation of this toxic effect in the presence of known amounts of ether vapor. It is suggested that cyclopropane be used to anesthetize patients receiving neomycin parenterally.

This work was supported by grant-in-aid No. B-1203 from the United States Public Health Service.

The authors acknowledge the technical assistance of Mr. Floyd Oppenheimer and the cooperation of the staff of the Department of Surgery, U. C. L. A. Medical Center. Dr. William Whalen supplied the closed chambers used in these studies and much helpful advice. The drugs used were supplied by Burroughs Wellcome & Co., Bristol Laboratories, and the Upjohn Company.

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