fraction does not function through a diaphorase would seem unlikely. Green(11) has demonstrated that the respiratory chain of beef heart mitochondria tends to fragment into several particles each of which contains several characteristic enzymes. In view of these findings it seems possible that certain of the LDH fractions, as demonstrated in starch gel, may consist of enzyme units. This is further suggested by the finding that the major diaphorase bands also contain malic dehydrogenase.

It is apparent that additional explanations for the electrophoretic heterogeneity of LDH must be considered before accepting the postulation that this heterogeneity represents different molecular species(1). Such possibilities as sulfhydryl or other protein interaction (12) or coupling of the dehydrogenase to various components of the respiratory chain cannot be excluded.

Summary. 1. An improved method for demonstration of lactic dehydrogenase after starch gel electrophoresis is described. This method employs a more sensitive tetrazolium and phenazine methosulfate. 2. Rat kidney contained 5 lactic dehydrogenase fractions which varied in cofactor requirements. DPN diaphorase was not demonstrable in any of these fractions, when reduced DPN was employed as the substrate, but was demonstrated in 2 additional bands. 3. The possibility is discussed that at least certain of the lactic dehydrogenase fractions from rat kidney exhibit electrophoretic heterogenity because of their association with various components of the respiratory system.

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Neuromuscular Blocking Properties of Various Polypeptide Antibiotics.† (26154)

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The neuromuscular blocking action of several antibiotics has been reported. Pittinger and associates(1,2) demonstrated the neuromuscular blocking action of neomycin sulfate and the enhancement of this effect by ether anesthesia. Brazil and Corrado(3) reported the "curariform" action of streptomycin. Timmerman and co-workers(4) reported neuromuscular blockade following administration of dihydrostreptomycin sulfate, polymyxin B sulfate and kanamycin sulfate. This report describes the neuromuscular blocking action of several polypeptide antibiotics and the effect of neostigmine methylsulfate and calcium chloride on it. Various constituents of the polypeptides were also evaluated for neuromuscular blocking activity.

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TABLE I. Chemical Composition and Neuromuscular Blocking (N.M.) Activity.

Antibiotic	Chemical composition*	ED_{50}, mg/kg (95% C.I.)	N.M. potency relative to colistin (95% C.I.)
Colistin	+ 6-methyloctanoic acid, D-leucine, L-threonine, L-leucine, L-α-γ diaminobutyric acid	9.1 (9.0- 9.2)	1.00 (——)
Polymyxin A	+ 6-methyloctanoic acid, D-leucine, L-threonine, L-α-γ diaminobutyric acid	14.0 (13.0-14.2)	.66† (.58- .70)
" B	+ 6-methyloctanoic acid, D-leucine, L-threonine, D-phenylalanine, L-α-γ diaminobutyric acid	5.7 (5.3- 6.0)	1.59 (1.53-1.64)
Neomycin	Diaminohexose moietie linked to D-ribose linked to neamine	34.2 (29.3-35.6)	.28 (.23- .32)
Viomycin	Creatinine, guanidino component, L-serine, α-β diaminopropionic acid	77.5 (77.1-78.0)	.12† (.09- .16)

* See ref. 7 and 9. † X^2 test for homogeneity of variance was non-significant at $p = .1\%$.

Methods. The following antibiotics were investigated: bacitracin, colistin sulfate, neomycin sulfate, polymyxin A sulfate, polymyxin B sulfate, ristocetin, tyrothricin, vancomycin hydrochloride, viomycin sulfate and sodium colistin methanesulfonate.* Solutions were freshly prepared and administered intravenously.

Neuromuscular blocking activity was evaluated using the sciatic nerve-gastrocnemius muscle preparation in the rabbit and chicken. Rabbits weighing 1.0-1.5 kg were anesthetized with sodium phenobarbital (200 mg/kg) and bipolar silver electrodes were placed on the peripheral end of the sectioned sciatic nerve. The nerve was stimulated for 0.2 sec with monophasic square wave pulses of 2 milliseconds duration at a frequency of 250 per sec and of supramaximal voltage delivered every 5 sec. A Grass stimulator (Model S4C) with circuit interrupter was used in all experiments. Semi-isometric recordings were made on smoked kymograph paper. Twelve to 19 rabbits were used to evaluate relative potency of each antibiotic. The dose producing 50% neuromuscular blockade (ED_{50}), potency ratios and their 95% confidence intervals were calculated as outlined by Finney (5) for unsymmetrical bioassays.

The effect of neostigmine methylsulfate (50 μg/kg) and calcium chloride (60 mg/kg) upon the neuromuscular blockade produced in rabbits was investigated. Antibiotics that produced blockade which was enhanced by neostigmine methylsulfate were studied in the chicken using the method described by Pelikan *et al.*(6). The parameters described for the rabbit sciatic nerve-gastrocnemius muscle preparation were also used in this preparation.

Various constituents of the polypeptide antibiotics producing neuromuscular blockade were also investigated in the rabbit sciatic nerve-gastrocnemius muscle preparation.

Results and discussion. Bacitracin, ristocetin, tyrothricin, vancomycin hydrochloride and sodium colistin methanesulfonate were administered to at least 2 rabbits at a dose of 100 mg/kg (except for bacitracin and tyrothricin, of which 25,000 units and 0.5 mg/kg, respectively, were given) and no neuromuscular blockade was observed. Colistin sulfate, neomycin sulfate, polymyxin A sulfate, polymyxin B sulfate and viomycin sulfate produced neuromuscular blockade as described below. The constituents of antibiotics studied, ED_{50} and relative potency of the active antibiotics are shown in Table I.

Colistin sulfate. This antibiotic recently introduced in Japan as Colimycin is structurally related to the polymyxins. For purposes of assessing relative potencies, colistin sulfate was chosen as the standard and therefore assigned a value of 1.00. The ED_{50} was 9.1 mg/kg. Neostigmine methylsulfate

* Colistin sulfate and sodium colistin methanesulfonate were kindly supplied by Warner Lambert Labs. Polymyxin A and + 6-methyloctanoic acid were obtained through the courtesy of Wellcome Research Labs.

did not appear to be an effective antagonist of the neuromuscular blockade produced by colistin sulfate. Likewise, calcium chloride did not antagonize the blockade. Colistin sulfate neither produced contracture when evaluated in the chicken nor was the blockade antagonized by 50 μg/kg of neostigmine methylsulfate. The methylated form of colistin (sodium colistin methanesulfonate) is devoid of neuromuscular blocking activity. This compound has been reported to be rapidly eliminated by the kidneys(7), which may explain in part its lack of activity.

Neomycin sulfate. The relative potency for neomycin sulfate was found to be 0.28. ED_{50} was 34.2 mg/kg. It has been reported (1,2) that calcium chloride as well as neostigmine methylsulfate is an effective antagonist of the neuromuscular blockade produced by neomycin sulfate. Our findings confirm these reports. Furthermore, successful clinical application of the antagonists has been reported (8).

Polymyxin A Sulfate. Polymyxin A differs chemically from polymyxin B in that it lacks D-phenylalanine (Table I). Relative potency for this compound was found to be 0.66. Its ED_{50} was 14 mg/kg. Neither calcium nor neostigmine methylsulfate were effective antagonists of the neuromuscular blockade produced by polymyxin A. Polymyxin A produced, in the chicken, a flaccid paralysis which was not antagonized by neostigmine methylsulfate.

Polymyxin B sulfate. This antibiotic was the most potent of the polypeptides studied. Relative potency and ED_{50} were found to be 1.59 and 5.7 mg/kg, respectively. Neither neostigmine methylsulfate nor calcium chloride were effective antagonists. As with polymyxin A, a flaccid paralysis was produced in the chicken which was not antagonized by neostigmine methylsulfate.

Viomycin sulfate. Of the compounds studied, viomycin sulfate appeared to be the least active. Relative potency and ED_{50} were found to be 0.12 and 77.5 mg/kg, respectively. The antagonistic effect of neostigmine methylsulfate upon the neuromuscular blockade produced by viomycin sulfate is shown in Fig. 1. Calcium chloride was also found to be an effective antagonist of the blockade produced.

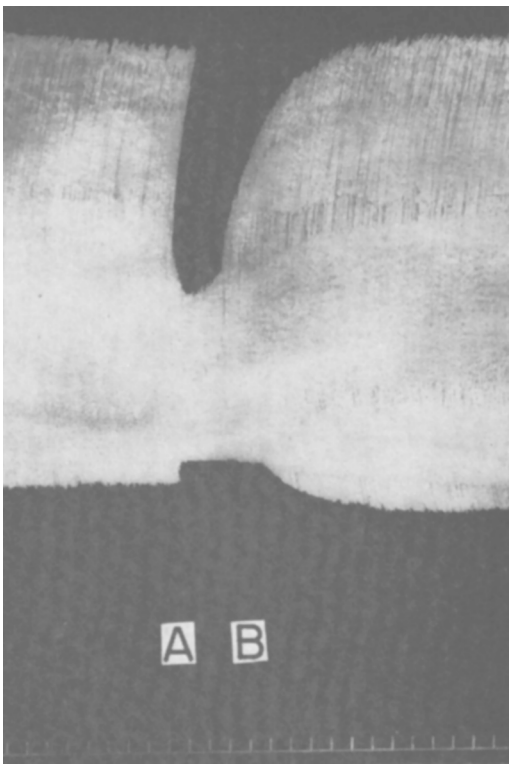

FIG. 1. Effect of neostigmine methylsulfate upon neuromuscular blockade produced by viomycin sulfate. Time marks equal 1 min. A. Viomycin sulfate, 90 mg/kg. B. Neostigmine methylsulfate, 50 μg/kg.

The various amino acids and other constituents comprising the antibiotics (Table I) were also investigated in the rabbit for neuromuscular blocking activity. The following compounds failed to produce neuromuscular blockade when evaluated using doses up to 100 mg/kg: D-leucine, L-threonine, L-leucine, α-γ diaminobutyric acid, D-phenylalanine, creatinine and L-serine. +6-Methyloctanoic acid when evaluated in doses up to 10 mg/kg showed no effect.

The preceding data demonstrate the neuromuscular blocking properties of several polypeptide antibiotics. The neuromuscular blockade produced by these agents was reversible and duration of action was dose related. Neuromuscular blockades of 85-95% returned to control levels in 20-40 minutes. The results obtained with various antagonists

suggest that the polypeptides studied do not have the same mechanism of action. Neostigmine methylsulfate or calcium chloride were not effective antagonists of the neuromuscular blockade produced by polymyxin A sulfate, polymyxin B sulfate and colistin sulfate. However, these compounds were effective antagonists of the blockade produced by neomycin sulfate or viomycin sulfate.

The failure of the polypeptide components to produce neuromuscular blockade suggests that the intact peptide molecule is necessary for activity. If there is a critical distance between 2 nitrogen atoms necessary for neuromuscular blockade, as suggested by various investigators(10,11,12) then one might expect the necessity of the intact peptide molecule or at least more than a single amino acid to be necessary for neuromuscular blockade.

We are unaware of any clinical reports of respiratory depression resulting from the neuromuscular blocking action of these agents other than neomycin sulfate. However, it is conceivable that the other antibiotics could produce undesirable clinical effects under circumstances of overdosage, excessive absorption, or in presence of anesthetic agents capable of enhancing their neuromuscular blocking action.

Summary. Colistin sulfate, neomycin sulfate, polymyxin A sulfate, polymyxin B sulfate, and viomycin sulfate produced neuromuscular blockade when tested on sciatic nerve-gastrocnemius muscle preparations of the rabbit. Data obtained from the neuromuscular preparations were statistically analyzed and the relative potencies of the antibiotics in relation to colistin sulfate were determined. Polymyxin B sulfate was the most active antibiotic, approximately 1.5 times as active as colistin sulfate. Neostigmine methylsulfate antagonized the neuromuscular blockade produced by neomycin sulfate and viomycin sulfate, but was not an effective antagonist of the blockade produced by colistin sulfate, polymyxin A sulfate, and polymyxin B sulfate. Colistin sulfate, polymyxin A sulfate and polymyxin B sulfate do not produce contracture when administered to the chicken. The amino acids and other constituents comprising the various peptide antibiotics failed to show neuromuscular blocking activity.

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