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effects were completely missing when the dosis remained below the 15 fold daily therapeutic dose for men.

2. The renal damage is always limited to the tubules of the cortex; regeneration and return or function of cor use vary rapidly

3. The extend of the gastric and renal damage seams to be parallel to the blood level of the compound.

4. Long termed subcutaneous administration of 500 000 units pro kg colistin-methane-sulfonate does not lead to any disturbance of vestibular function in mice.

As far as it is permissable to draw conclusions for the use in men, may be said, that, with respect to side effects, colistin-methanesulfonate compares comparably with compounds of neomycin group.

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Reciprocal Potentiation between Succinylcholine and Colistin in the Neuromuscular Blockade

Vital-Brazil and Corrado (1), while studying the mechanism of respiratory paralysis, found that streptomycin administered in toxic doses to dogs produced a blocking effect at the neuromuscular junction.

Vital-Brazil (2, 3) and other workers (4) confirmed later such discovery and established the conditions for the phenomenon to take place, its reversibility by calcium chloride and the dose-effect relationship. Later, Corrado and Ramos (5—7) and some other authors (8, 9) demonstrated that other antibiotic chemically related to streptomycin, such as, neomycin and kanamycin produced the blocking effect of the neuromuscular transmission as well as blockade at the level of neurovegetative ganglions. Recently, Vital-Brazil and coworkers (10) established that viomycin, an antibiotic composed essentially of aminoacids, also produced the neuromuscular blocking effect.

With this background, it was considered of interest to investigate if colistin, a cyclic peptide antibiotic, produces the same effect as the above mentioned antibiotics.

Materials and Methods

Two series of experiments were done: the first one was designed to study the influence of colistin on the neuromuscular transmission and the second one, dealt with both the toxicity of this substance and the combined toxic effect of colistin and succinylcholine. For the first series,

male guinea pigs of 480 to 600 gm body weight were used. The animals were anesthetized with a mixture of urethane (600 mg/kg) and sodium pentobarbital (10 mg/kg) injected by intraperitoneal route. The trachea was cannulated to give, if necessary, artificial respiration and a polietylene tube was inserted in the external jugular vein in order to inject the drugs. Sciatic nerve-tibialis anterior muscle preparation was set up in the conventional manner using a Brown-Schuster myograph. The tendon of the tibialis anterior muscle was attached to a flat spring and the muscle contractions were recorded on a smoked paper. Platinum electrodes were placed both on the sciatic nerve branch which innervate the tibialis anterior muscle and directly on the muscle. Square waves of 0.2 msec. duration and a frequency of 60 per minute delivered from an electronic stimulator (American Electronic Labs., Inc., model 751) were used to excite the preparation. In most of the experiments, the nerve and the muscle were alternatively excited but in a few essays the two legs were prepared, one for direct and the other for indirect stimulation and were excited simultaneously. In each animal, the threshold and the strength required to cause the maximal twitch for the direct as well as for the indirect stimulation were previously studied.

For the essay with the drugs, except in the series corresponding to the colistin alone, a standard stimulation twice that required for the maximal twitch was used. Each type of experiment was repeated, at least, in five animals.

In the first group of animals, only the effect of colistin was studied, testing increasing doses of up to 1000 mg/kg from a batch which contained 13 500 U/mg. Stimuli lower than twice the maximal were used.

The mutual influence between colistin and succinylcholine was studied in the next two groups of guinea pigs: a) succinylcholine was injected first in a standard dose of 50 mcg/kg intravenously. Then, 20 minutes later, 500 mg/kg of colistin was administered also by intravenous route and after 30 minutes a second dose of succinylcholine was given, and b) the sequence of drugs was: colistin first, then 30 minutes later succinylcholine and finally 15 minutes later the second dose of colistin was administered, always in the standard doses, respectively.

The second series of experiments war carried out on male adult white mice of 20 to 22 gm body weight. Colistin was injected by intraperitoneal route and succinylcholine intravenously. The LD_{50} of the two drugs was first studied separately and then their combined action was tested by administering colistin followed by toxic doses of succinylcholine. Colistin was used as the metansulphonate and succinylcholine as the chloride. The doses given corresponded to these salts.

Results

1. *Effects of colistin.* — Using threshold stimulations for the muscle, as well as for the nerve and doses of colistin of 100 mg/kg, a decrease in the muscle contractions amplitude was observed which amounted to 30% and 15% by the direct and the indirect stimulations, respectively. The inhibition started 5 minutes after the injection and it reached the maximum at 15 minutes. With a dose of 500 mg/kg, the inhibitions were 70% and 21%, respectively, and the maximum effect appeared after 25 minutes of aministering the drug.

Tab. 1. *Inhibition of the muscular contraction produced by Colistin*

Doses	% of inhibition	
	Indirect stimulation	Direct stimulation
300 mg/kg	13.2	9.2
500 "	38.3	18.3
1.000 "	55.4	31.4

Using the suprathreshold stimulation of the muscle for both the muscle and nerve, it was found that with doses lower than 250 mg/kg, there was no inhibition of the muscular contraction. At higher doses, colistin produced inhibition. The greater the doses, the higher was the effect (Tab. 1). The peak effect appeared after 20 minutes.

When the standard stimulation twice the strenght required to cause the maximal twitch of the muscle was used, colistin by itself (Fig 1A) did not produce any decrease of the muscular contraction neither under the direct stimulation, nor under the indirect one even with individual doses of 1 gm/kg. The stimulations were repeated with doses of up to 2 gm/kg.

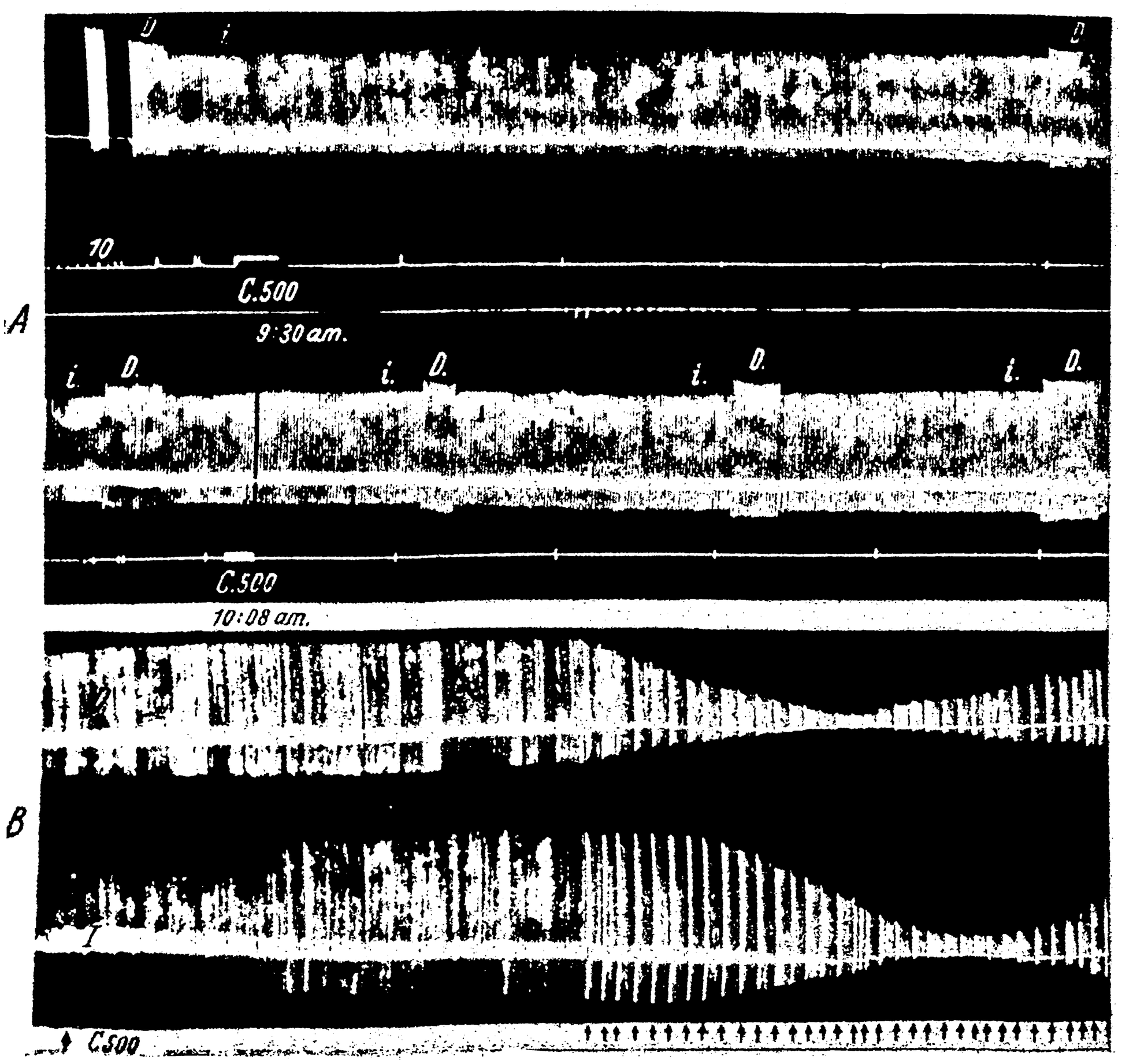

Fig. 1. Effect of colistin alone (A) and after succinylcholine (B). A. Guinea pig 480 gm. B, guinea pig 490 gm. Maximal twitches of the tibialis anterior muscle were elicited directly (D) and indirectly (I) once every second. In A, alternative recordings and in B simultaneous recordings for both legs. C500 = colistin, 500 mg/kg, i—v. In A, repeated doses at 38 minutes interval; in B, one dose after 20 minutes of the injection of 50 mcg/kg of succinylcholine. Time, 1 minute. In B, each arrow, 1 minute.

2. *Potentiation of the succinylcholine effect.* — Succinylcholine, by itself, produced total blockade of the neuromuscular transmission, though of short duration in only 4 out of 8 animals (Fig. 2A). In the other 4, it produced partial blockade (Fig. 2B). There was also inhibition of the muscular contraction, though it was not too strong, when the muscle was directly stimulated. On the other hand, when the same dose of succinylcholine was administered 20 minutes after the administration of colistin, it produced a complete neuromuscular blockade of a duration 15.3 times longer (Fig. 2C and Tab. 2). When in the control series, a second dose of succinylcholine was given at the same interval but without the injection of colistin, the extent of the inhibition as well as its duration was very similar to that produced by the first dose.

Small doses of succinylcholine such as 10 mcg/kg did not produce any neuromuscular blockade in the test essays, although after colistin they produced a 60 to 85% inhibition (indirect stimulation) which lasted from 12 to 15 minutes.

3. *Potentiation of the colistin effect.* — Colistin, administered before succinylcholine, according to that was formerly described, did not produce either inhibition of the muscular contraction nor neuromuscular blockade. Instead, when it was given up to 20 minutes after a previous dose of succinylcholine, it produced 77% and 60% inhibition of the muscular contraction elicited by indirect and direct stimulation, respectively (Fig. 1B). Inhibition lasted approximately 24 minutes. Due to the fact that colistin given at longer than 1 hour intervals, promoted cumulative effects, for this series of experiments two different group, of animals were used: one receiving only colistin and the other one receiving colistin following a dose of succinylcholine. The inhibition effect appeared and lasted in the same way, 2 to 3 minutes. Then, it was progressive until approximately 25 minutes after the injection of colistin. The recuperation was likewise, slow.

4. *Toxicity potentiation.* — Prior administration of colistin enhanced succinylcholine toxicity. As it can be seen in Fig. 3, to a greater colistin dose corresponded a greater succinylcholine toxicity and its DL_{50} decreased. The DL_{50} of colistin (i-p) was 518±22 mg/kg. Doses lower than 300 mg/kg did not produce mortality. The DL_{50} of succinyl-

Tab. 2. *Inhibition of the muscular contraction produced by succinylcholine (50 mcg/kg). Intensity and duration of the effect before and after a dose of 500 mg/kg of colistin.*

	Stimulation	
	Indirect	Direct
% of inhibition:		
Before	84	28
After	100	66
P*	<0.05	<0.01
Duration of the maximal inhibition:		
Before	3.1'	1.2'
After	47.5'	40.3'
P*	<0.01	<0.01

* Probability value of the statistical significance between the values „before" and „after"

choline produced 100% of mortality after a dose of 100 mg/kg of colistin. Using the DL_{25} of succinylcholine after variable intervals of colistin (100 mg/kg), it was found that at 15 minutes the mortality was only 25%; at 30 and 60 minutes, 60%; at 120 minutes 40%. This revealed that the greatest activity of colistin took place between 30 and 60 minutes after its administration.

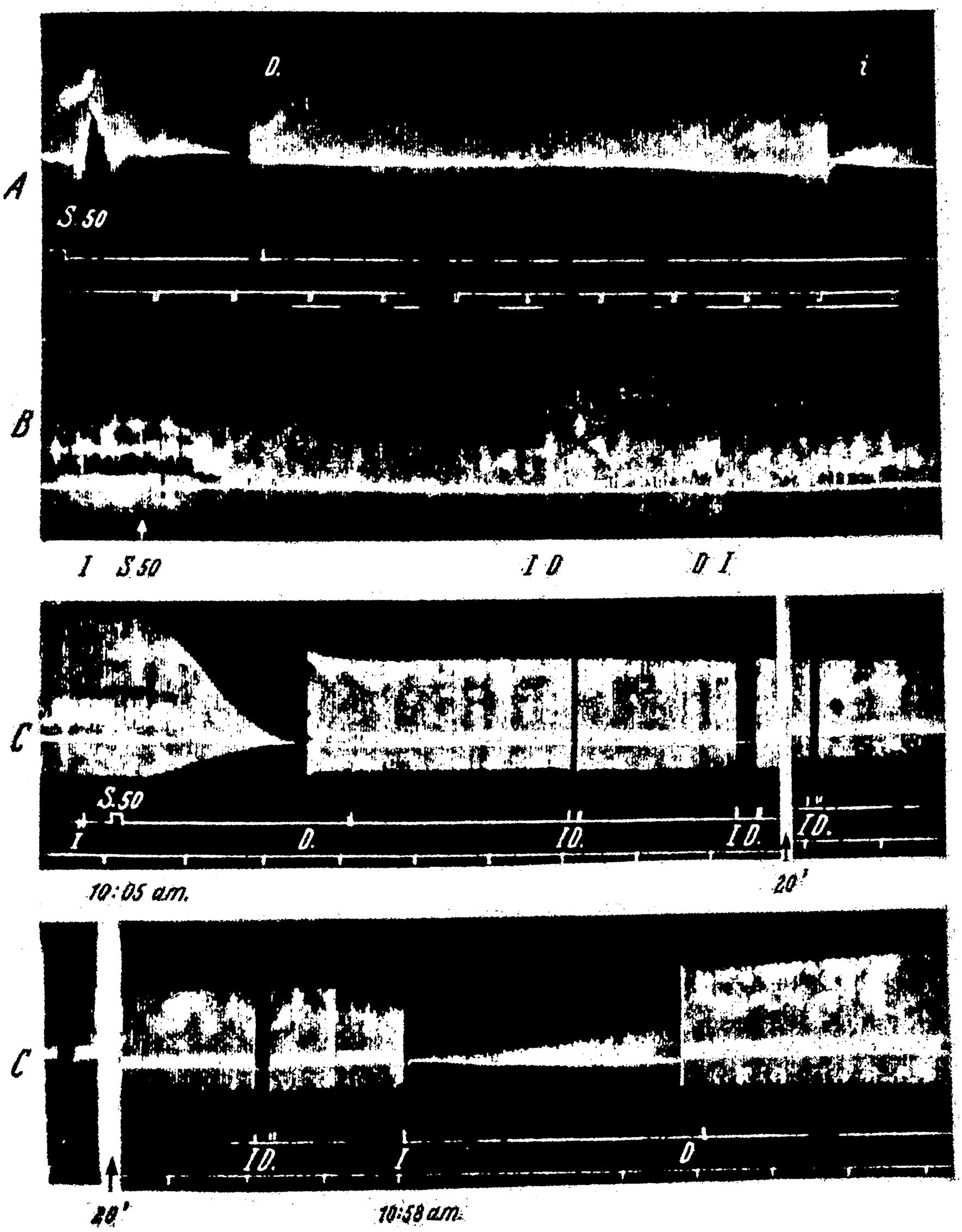

Fig. 2. *Effect of succinylcholine before (A and B) and after (C) colistin.* Guinea pigs 480—500 gm. Maximal twitches of the tibialis anterior muscle were elicited directly (D) and indirectly (I) once every second, alternative recordings.
S50 = succinylcholine, 50 mcg/kg, i—v. In A and B, total and partial neuromuscular blockade, respectively.
In C, the same dose after 20 minutes of the administration of colistin. Time, 30 seconds.

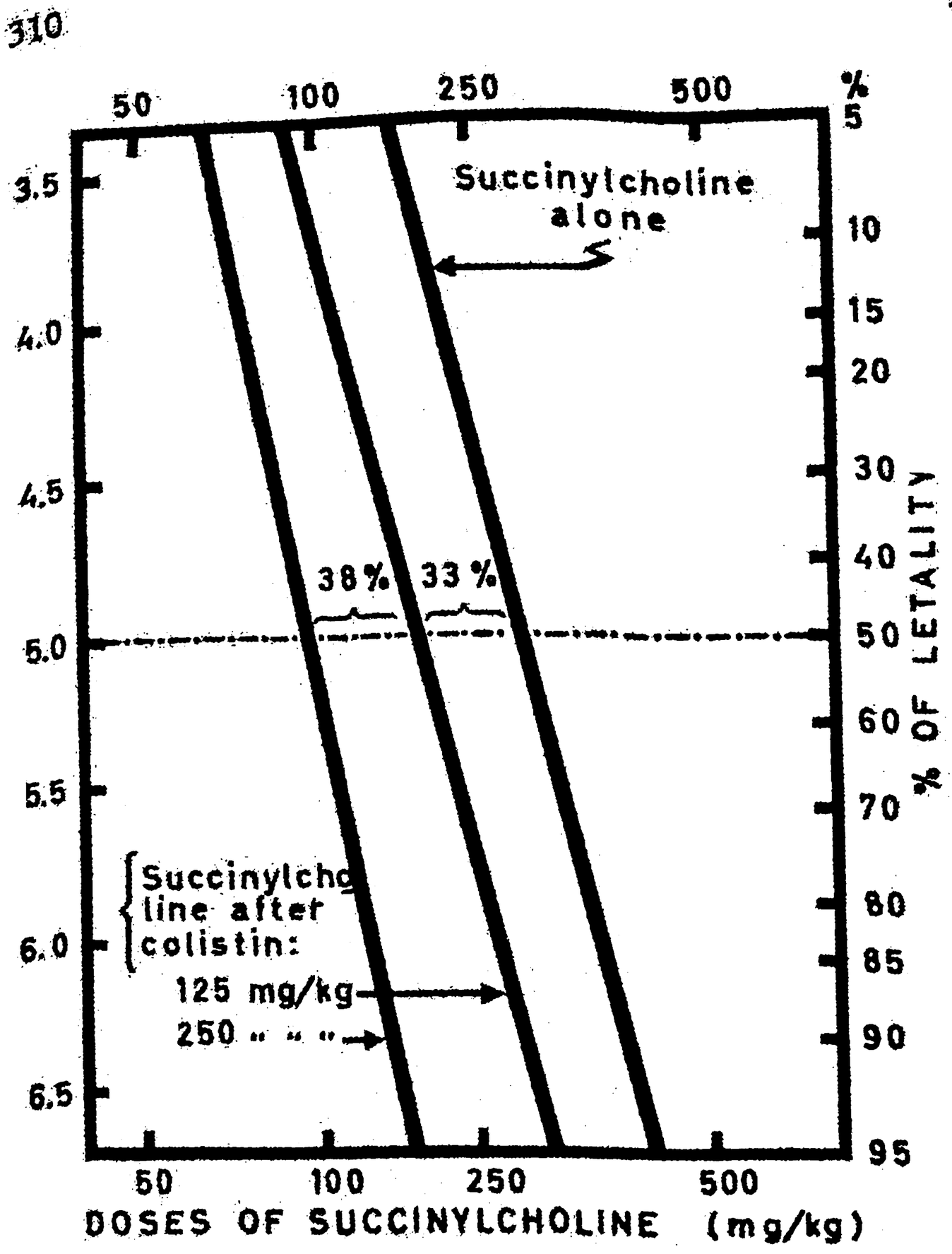

Fig. 3. *Increase of succinylcholine toxicity after colistin administration.* White mice. Each regression line was based on an experiment with 50 animals, 10 for each dose.

Discussion

The results revealed that colistin in very high doses is capable of inhibiting the muscular contractions caused by direct stimulation and above all by indirect stimulation. Its potency as blocking agent of the neuromuscular transmission would be quite inferior to that of streptomycin, neomycin and kanamycin (2, 5–9). The most outstanding fact in the present investigation which apparently has not been observed by other investigators who have worked with other antibiotics, is the mutual potentiation of the muscular blocking effect which was observed between the colistin and succinylcholine as well as the potentiation of the toxicity of succinylcholine by colistin. This fact might be of clinic importance if the phenomenon is also produced in human beings, particularly in patients who being under treatment with this antibiotic, might undergo surgery. In such situation, it will be necessary to administer reduced doses of succinylcholine.

Zusammenfassung

Nach der i.v. Injektion von Colistin in Dosen von 100 mg/kg findet sich eine geringe Verminderung der Amplitude der Kontraktionen des M.tibialis anterior des Meerschweinchens. Dieser Effekt wurde aber nur erzielt, wenn die Reizungen sich im Schwellenbereich bewegten.

Die Hemmung war größer bei der direkten als bei der indirekten Reizung, mit höheren Dosen von Colistin erhöhte sich die Intensitä und Dauer der Kontraktionen. Der Hemmungseffekt verminderte sich, wenn stärkere Reize verabfolgt wurden, vor allem bei direkter Reizung. Wenn die Reizstärke auf das zweifache der Maximalkontraktion gesteigert wurde, blieb die Hemmung sogar bei Dosen von 1 g/kg aus. Nach Verabfolgung von Succynilcholin blockiert Colistin teilweise die neuromuskuläre Übertragung, und eine vorherige Verabreichung von Colistin führt zu einer beträchtlichen Erhöhung der Intensität und Dauer des neuromuskulären Succinylcholinblockeffektes. Niedrige Dosen von Succynilcholin die allein keinen neuromuskulären Block auslösten, führten zu komplettem Block, wenn vorher Colistin gegeben wurde. Ebenso erhöhte sich die Toxizität von Succynilcholin durch vorherige Verabreichung nicht letaler Dosen von Colistin. Die größte Toxizität fand sich 30 bis 60 Minuten nach der Colistin-Iniektion.

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Die parenterale Colistin-Therapie; Wirkstoffspiegel und antibakterielle Aktivität

Von den zahlreichen Faktoren, die den Erfolg oder Mißerfolg einer Therapie am Krankenbett bestimmen, sind einmal die antibakterielle Aktivität des Antibiotikums, zum anderen die im Patientenorganismus realisierbaren Wirkstoffkonzentrationen einer relativ exakten Messung und Beurteilung zugänglich. Diese Daten sind die entscheidenden Kriterien für die Beurteilung der Erregerempfindlichkeit und sind zugleich unentbehrlich für eine Dosierung, die sich an der Sensibilität des vorliegenden Erregers orientiert. Während über die antibakterielle Aktivität des Colistin eine ganze Reihe von Ergebnissen vorliegen, ist dem Kliniker, Chemotherapeuten und Bakteriologen eine Orientierung über die in vivo erreichbaren Colistin-Konzentration außerordentlich erschwert. Die von verschiedenen Autoren im Schrifttum mitgeteilten Resultate von Blut- und Liquorspiegelbestimmungen sind infolge einer bunten Vielfalt der Definitionen und Begriffe, der Präparationen und Dosierungen kaum noch vergleichbar und nicht mehr zu einer einheitlichen Aussage zusammenzufassen. Der eine Untersucher verwendet Colistin als Sulfat, andere als Chlorhydrat oder Methansulfonat, wobei die Auswertung beim einen

Autor gegen homologen Standard, beim anderen jedoch gegen Colistin-Sulfat erfolgt. Definitionen in mcg Base, mcg Sulfat, Methansulfonat und Chlorhydrat konkurrieren mit biologischen Einheiten, die – um die Verwirrung voll zu machen – sowohl als japanische als auch als britische Einheiten interpretiert werden (2, 3, 4, 7, 11, 12, 13, 16).

Es war daher naheliegend, eigene Spiegelbestimmungen im Blut und Liquor cerebrospinalis durchzuführen und an einem größeren Kollektiv gram-negativer Erreger die antibakterielle Aktivität von Colistin zu bestimmen. Aus der Korrelierung der ermittelten Daten soll eine prinzipielle Wertbemessung des Colistin für die Therapie von Pyocyaneus- und Coli-Infektionen abgeleitet werden.

Da aus Gründen der besseren Verträglichkeit für die parenterale Applikation nur das Sulfomethyl-Derivat des Colistin Verwendung findet, wurden alle Untersuchungen ausschließlich mit Colistin-Na-methansulfonat durchgeführt[1]). Sämtliche Angaben werden dabei auf den Gehalt an wirksamer Base bezogen, erfolgen also gravimetrisch in mcg oder mg Base. Lediglich bei der Dosierung wird daneben auch noch die Bezeichnung in japanischen Einheiten (1 E = 0,0333 mcg Base) beibehalten, um einen Vergleich mit den in Einheiten üblichen Dosierungsangaben der therapeutischen Praxis zu ermöglichen.

Die Spiegelbestimmungen wurden im Lochplattenverfahren (Agardiffusionstest) mit Bordetella bronchiseptica ATCC 4617 als Teststamm durchgeführt. Die unterste Nachweisgrenze lag bei etwa 0,1 mcg Base/ml. Die Auswertung erfolgte gegen Colistin-Na-methansulfonat, also gegen homologen Standard. Ansatz der Standard-Verdünnungen in menschlichem Serum, das zuvor auf eventuelle antibakterielle Eigenaktivität geprüft war.

Bei den Aktivitätsbestimmungen an 200 Stämmen von Pseudomonas aeruginosa sowie 50 Stämmen von E. coli und coliformen Keimen im quantitativen Röhrchenverdünnungstest wurde ebenfalls Colistin-Na-methansulfonat der gleichen Charge verwendet. Die minimalen Hemmkonzentrationen (MIC) sind in mcg Base/ml angegeben. Die bakterizid wirkenden Konzentrationen wurden durch Subkultur der klar gebliebenen Teströhrchen ermittelt und sind in jedem Fall als „totale Bakterizidie" zu verstehen.

Die Spiegelbestimmungen im Serum erfolgten an insgesamt 23 erwachsenen Probanden jeweils 2, 4 und 6 Stunden nach einmaliger intramuskulärer Injektion von 1 bis 4 Mega-Einheiten (33 bis 133 mg Base) in Form des Colistin-Na-methansulfonat. Liquorspiegel wurden bei 10 erwachsenen Patienten ohne entzündliche Veränderungen der Meningen durchgeführt, und zwar ebenfalls 2 bis 4 Stunden nach einmaliger intramuskulärer Applikation von 2 bis 4 Mega-Einheiten Colistin-Na-methansulfonat (66 bis 133 mg Base). In jedem Fall wurde zugleich auch der entsprechende Serumspiegel bestimmt.

Die Resultate unserer Blutspiegelbestimmungen sind detailliert in den Tab. 1 bis 4 und als Mittelwertkurven von insgesamt 23 Probanden in Abb. 1 wiedergegeben. Leider ist ein quantitativer Vergleich mit den Ergebnissen anderer Untersucher aus den schon erwähnten Gründen nicht möglich und verbietet sich speziell wegen der differenten Standardsubstanzen, bei denen Diffusions- und Aktivitätsunterschiede zwischen Standard und Test nicht auszuschließen sind (12, 16).

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[1]) Colistin-Na-methansulfonat wurde als therapeutische Substanz sowie in analytischer Einwaage (25,93 mg/Ampulle) als Standardsubstanz mit einem Milligramm-Titer von 11 569 E/mg (Charge Nr. 509, F = 2,593) liebenswürdigerweise von der Fa. Chemie Grünenthal GmbH, Stolberg, zur Verfügung gestellt.