emphasize the differences between the local actions of these drugs on hippocampal and neuromuscular transmission.

The excitatory effects of dTC in several areas of the central nervous system have been attributed primarily to the blocking of inhibitory synapses(11,12). In the cortex the local synchronous discharges produced by topical dTC were likened to strychnine spikes (13) and according to the schema developed by Grundfest(14) dTC would thus act postsynaptically to inactivate inhibitory synapses. On the other hand, in the hippocampus, the characteristics and waveform of the hypersynchronous discharges, as well as the marked potency of dTC, were more analogous to the foci previously described by our group for picrotoxin than for strychnine(8). This would suggest that dTC acts similar to picrotoxin in the hippocampus by interfering with presynaptic inhibitory regulatory mechanisms, thus functioning as a disinhibitor. In fact, it had been proposed that dTC preferentially produces microlesions at presynaptic sites(15). It is reasonable to expect that the pharmacological lesion produced with dTC will offer us another useful tool for analyzing the complex chemical processes that govern local hippocampal excitability.

Summary. Of a series of neuromuscular blocking agents microinjected into the hippocampus, only d-tubocurarine (dTC) was highly selective in establishing a focus at injection sites. These findings coupled with the ineffectiveness of cholinomimetics to antagonize the dTC focus emphasize the differences between the actions of neuromuscular blockers on hippocampal and neuromuscular transmission. Potency of dTC and characteristics of the focus also suggest that dTC acts similar to picrotoxin by interfering with presynaptic inhibition.

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Production of L Forms of *Neisseria meningitidis* by Antibiotics. (31804)

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Previous reports have demonstrated that L forms of certain bacteria are produced by antibiotics that inhibit bacterial cell wall synthesis(1-3). Four such antibiotics that have been employed for L form production are penicillin, methicillin, cycloserine and cephalothin. More recently bacitracin has been reported to produce L forms of group

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A streptococci(4). Though biochemical studies have suggested that ristocetin and vancomycin also inhibit cell wall synthesis (5-7), L form production by these antibiotics has not been previously described(1,8).

Recently, L forms of *Neisseria meningitidis* were produced by the penicillin gradient plate technique(9). This report describes the production and cultivation of meningococcal L forms in the presence of methicillin, ampicillin, cycloserine, cephalothin, ristocetin, bacitracin and vancomycin. Certain characteristics of L forms produced by each antibiotic and of their respective revertant organisms are compared.

Materials and methods. The group B meningococcal strains used in this study have been previously described(9). Parent and revertant meningococci were subcultured either in BBL Eugon broth or on Difco Mueller-Hinton chocolate agar. Broth cultures were placed on an Eberbach reciprocating shaker (6500 rev/hr) and incubated aerobically at 37°C for 12-18 hours. Cultures on agar were incubated at 37°C in the presence of moisture and CO_2 (candle jar) for 18-24 hours. The medium for production and cultivation of L forms was brain heart infusion (Difco) of pH 7.2-7.4 containing 1.2% agar, 10% sucrose, 0.5% yeast extract and 10% inactivated (60°C for 30 min) horse serum. Hereafter this medium is designated BRHIA. Plate cultures with L form colonies were incubated at 37°C in the presence of moisture and CO_2 (candle jar).

The method for production of L forms was the gradient plate technique(9). In addition to benzylpenicillin (Charles Pfizer and Co.), other antibiotics tested were methicillin and ampicillin (Bristol Lab.); cycloserine,† cephalothin and vancomycin (Eli Lilly and Co.); ristocetin and erythromycin (Abbott Lab.); novobiocin (Upjohn Co.); tetracycline (Lederle Lab.); bacitracin (Premo Lab.) and sulfisoxazole (Roche Lab.). L form cultures were serially transferred every 2-4 days on BRHIA containing the antibiotic responsible for their production.

† Cycloserine was kindly supplied by Dr. R. S. Griffith, Indianapolis, Ind.

TABLE I. Antibiotic Concentrations Used for the Production and Propagation of L Forms.

Antibiotic	Production	Propagation
units/ml		
Penicillin	100,000*	1,000†
Bacitracin	5,000	50
mg/ml		
Methicillin	500	1.0
Ampicillin	250	1.0
Cephalothin	250	.1
Cycloserine	10	1.0
Vancomycin	50	1.0
Ristocetin	25	1.0

* Concentration—per ml of diluent.
† Concentration—per ml of complete medium.

The concentrations of those antibiotics that produced and supported propagation of L forms are shown in Table I. Colony morphology (× 45 magnification) and carbohydrate fermentations(10) of L forms produced by each antibiotic were compared.

L form cultures were continued for 10 serial transfers. To allow reversion to the bacterial form, L form colonies from the 1st, 5th and 10th serial passage on media containing an antibiotic were transferred to antibiotic-free BRHIA. Subsequently each revertant strain was identified by serologic agglutination (macroscopic slide technique) and by carbohydrate fermentations(11). In addition, revertant meningococci derived from L forms which had had 10 serial passages were transferred to gradient plates containing each test antibiotic and the degree of L form growth noted.

Sensitivities to each test antibiotic were compared for the meningococcal L form, its parent and its revertant strain. Serial 10-fold dilutions of each antibiotic were prepared in distilled water and each dilution added to BRHIA. This medium was inoculated with either 0.2 ml of a 6-hour broth culture of parent or revertant organisms (10^4/ml) or with agar blocks containing a 2-day growth of L form colonies. These L forms had been produced with penicillin, and following 100 serial transfers on BRHIA with penicillin, non-reverting L form colonies were isolated. These stabilized L forms subsequently had at least 30 serial passages on penicillin-free BRHIA prior to sensitivity determinations. Plates inoculated with either

TABLE II. L Form Production from Parent and Revertant Miningococci by Exposure to Cell Wall Antibiotics.

Antibiotic	Parent strain				Revertant strain†			
	78	79	NE	55	78R	79R	NER	55R
Penicillin	+*	+	+	+	+	+	+	+
Methicillin	+	+	+	+	+	+	+	+
Ampicillin	+	+	+	+	+	+	+	+
Cycloserine	—	—	+	+	+	+	+	+
Cephalothin	—	+	—	+	+	+	—	+
Ristocetin	—	+	—	+	—	+	—	+
Bacitracin	—	—	—	—	—	+	—	—
Vancomycin	—	—	—	—	—	+	—	—

* Symbols: + = L form growth; — = No L form growth.
† Revertant strains from penicillin-induced L forms.

parent or revertant meningococci were read on the 1st, 2nd, and 5th day, whereas plates inoculated with L form colonies were read on the 2nd, 5th, and 10th day.

Results. Production of L forms. Preliminary studies of L form production from parent meningococci and from revertant strains of penicillin-induced L forms are shown in Table II. Parent bacteria did not produce L forms when exposed to bacitracin or vancomycin. Revertant strain 79R was the only strain from which L forms were produced by each of the 8 cell wall antibiotics tested.

Subsequent studies showed that revertant meningococci from L forms produced by any one antibiotic yielded a heavy growth of L forms when inoculated onto gradient plates containing that same antibiotic. Revertant strains from L forms produced by methicillin, ampicillin, cycloserine or cephalothin also yielded many L forms when exposed to any other of these 4 antibiotics. Only an occasional L form colony was produced when these revertants were exposed to ristocetin, bacitracin or vancomycin.

L forms were *not* produced when parent or revertant meningococci were exposed to novobiocin, tetracycline, erythromycin or sulfisoxazole. It was also noted that L form growth did not occur when meningococci were inoculated onto control plates without antibiotics.

Colony morphology and fermentative reactions of L forms produced by each antibiotic. For comparative studies, each antibiotic-induced L form from meningococcal parent strain 79 or revertant strain 79R was serially transferred on BRHIA containing the respective cell wall antibiotic (Table I). All L forms propagated well on these media. Fig. 1 shows the similarity of colony morphology of L forms produced by ristocetin and vancomycin. The other antibiotics produced colonies with identical morphologic characteristics. In general, each L form colony consisted of a distinct central area surrounded by a lighter periphery. Colony size was primarily determined by duration of growth and degree of crowding. All L forms produced acid from dextrose and maltose but not from sucrose.

Reversion of L forms and properties of revertant meningococci. Reversion of L forms readily occurred following 1 to 3 passages on antibiotic-free BRHIA, except in the case of L forms produced and propagated in the presence of ristocetin (see below). All revertant organisms from each antibiotic-induced L form produced acid from dextrose and maltose, but not from sucrose. Each revertant strain agglutinated only with group B meningococcal antisera. Revertant strains from ristocetin-, bacitracin-, and vancomycin-induced L forms produced coarse agglutination, whereas other revertant meningococci produced fine agglutination when added to group B meningococcal antisera. These agglutination patterns were not altered by repeated subculture of revertant organisms on chocolate agar.

To induce mass reversion, L forms which had had 1, 5 and 10 serial passages on ristocetin medium were transferred to media containing either 2.0 and 2.5% agar or 23% gelatin(12). Reversion of only those L forms which had had 1 passage was observed.

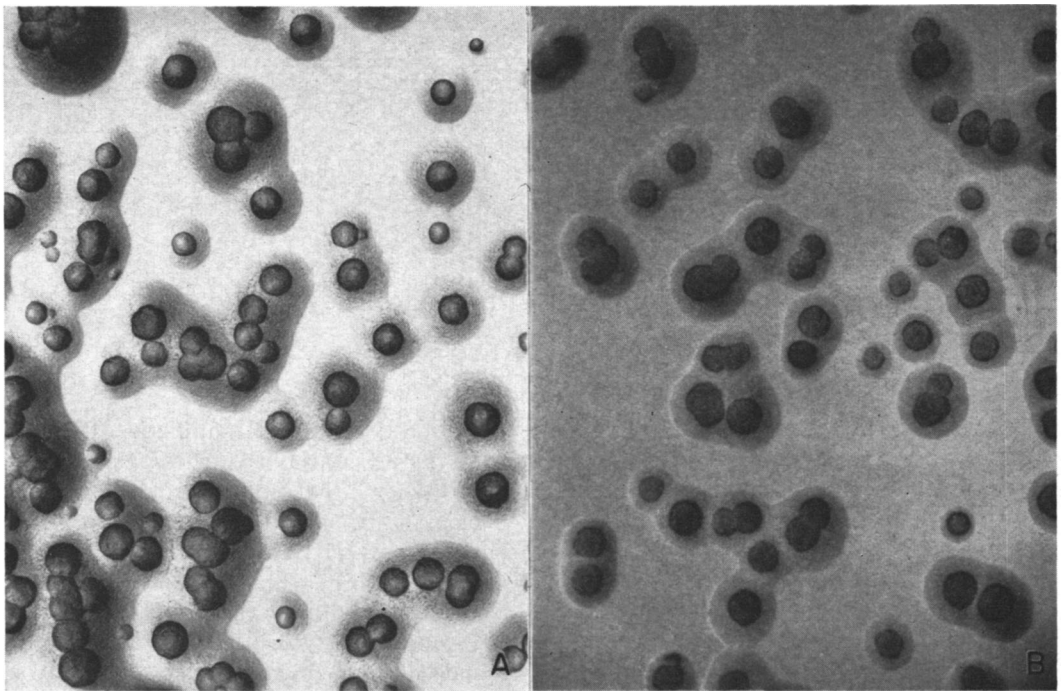

FIG. 1. Meningococcal L forms produced and propagated with (A) ristocetin and (B) vancomycin. L form colonies consist of a well-demarcated central area surrounded by a lighter periphery. (× 32).

Relative antibiotic sensitivities of L forms and parent meningococci. L forms were more resistant than parent strain 79 to all antibiotics that inhibit cell wall synthesis except ristocetin (Table III). Though resistant to ristocetin at a concentration of 1000 μg/ml, both L form and parent strain were inhibited by 2000 μg/ml. The sensitivities of both organisms to novobiocin, tetracycline and erythromycin were similar. Revertant meningococci and parent strain 79 had identical sensitivities to all antibiotics tested.

TABLE III. Relative Antibiotic Sensitivities of a Parent Meningococcus and Its L Form.

Antibiotic	Strain 79	
	Parent	L form
Units/ml		
Penicillin	1.0*	>10,000
Bacitracin	10	1,000
μg/ml		
Ampicillin	1.0	>10,000
Methicillin	10	>10,000
Cephalothin	10	1,000
Cycloserine	1,000	10,000
Vancomycin	1,000	10,000
Ristocetin	>1,000	>1,000
Novobiocin	1.0	1.0
Tetracycline	1.0	1.0
Erythromycin	1.0	1.0

* Minimal inhibitory concentration.

TABLE IV. Range of Antibiotic Concentrations Necessary for the Production of Meningococcal L Forms.

Antibiotic	Concentration					
	.1	1.0	10	100	1,000	10,000
units/ml						
Penicillin	B+L*	L	L	L	L	L
Bacitracin	B	B	L	L	—	—
μg/ml						
Ampicillin	B	L	L	L	L	L
Methicillin	B	B+L	L	L	L	L
Cephalothin	B	B+L	L	L	—	—
Cycloserine	B	B	B	B	L	—
Vancomycin	B	B	B	B+L	L	—
Ristocetin	B	B	B	B	B+L	—

* B = bacterial growth; L = L form growth; — = no growth.

Since antibiotic sensitivities were determined on a medium (BRHIA) that supported L form growth, these studies also demonstrated the range of antibiotic concentrations in which L forms of meningococci were produced. These results are shown in Table IV.

Discussion. L forms of meningococci were produced *in vitro* only by those antibiotics that inhibit bacterial cell wall synthesis. The production of L forms by ristocetin and vancomycin is in agreement with previous studies of the antibacterial action of these 2 antibiotics(5,6). Previous reports have suggested that the antibacterial action of ristocetin, bacitracin and vancomycin on cell wall synthesis may differ from that of penicillin(5, 13). In light of these studies, the present observations describing (a) differences in the production of L forms from revertants of various antibiotic-induced L forms, and (b) different patterns of group B agglutination of revertant meningococci are of interest. The possibility that these findings are in any way related to a difference in the antibacterial action of these antibiotics remains speculative.

Morphologic, fermentative and growth characteristics of L forms produced and propagated with each antibiotic were similar. The only apparent difference was that L forms produced and serially propagated with ristocetin failed to revert to the bacterial form when transferred to antibiotic-free medium.

L forms were more resistant than either parent or revertant organisms to 7 of the 8 antibiotics that inhibit cell wall synthesis, whereas sensitivities of parent and L form to 3 antibiotics that did not produce L forms were similar. Previous studies have shown that parent and L form are equally sensitive to sulfadiazine(9). The failure of sulfisoxazole to produce L forms *in vitro* is in accord with these sensitivity findings.

Previous reports have shown that the antibacterial action of cycloserine is competitively antagonized by alanine(14,15). The relatively large amount of this amino acid in complex media(16) may explain the low sensitivity values of parent and revertant strains to this antibiotic. The sensitivity values of these bacteria to the other antibiotics tested were similar to those reported by Eickhoff and Finland(17).

Summary. L forms of group B *Neisseria meningitidis* were produced by penicillin, methicillin, ampicillin, cephalothin, cycloserine, ristocetin, bacitracin and vancomycin. These L forms were propagated serially on medium containing each antibiotic, and all L forms had similar growth, morphologic and fermentative properties. Revertant organisms from each antibiotic-induced L form had the same fermentative properties and antibiotic sensitivities as the respective parent meningococcus. Furthermore each revertant strain agglutinated with group B meningococcal antisera. L forms were more resistant than the parent organism to those antibiotics that were capable of producing L forms, except in the case of ristocetin where parent and L forms exhibited the same sensitivity. Parent and L form organisms exhibited similar sensitivities to novobiocin, tetracycline and erythromycin, and L forms were not produced by these antibiotics or by sulfisoxazole.

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