ACh over their whole surface and this sensitivity is associated with a high myosin-ChE and low myosin-ATP'ase activity(10,11). Global chemosensitivity of muscle fibers in the newborn animal could satisfactorily account for contracture-inducing effects of succinylcholine reported here. There is, however, no satisfactory explanation as to why succinylcholine fails to evoke contractures in the cat after 10th-12th postnatal day. If this is related to the finding that at this time the chemosensitivity of muscle fibers is restricted to the end-plate region(4), then it must be allowed that the changing responsiveness of skeletal muscles in newborn cats to succinylcholine is not *immediately* related to establishment of motor innervation, but to some factors which are brought into operation during the latter phases of ontogenesis.

Summary. Intravenous succinylcholine produces generalized, prolonged contractures in newborn cats prior to 10th-12th postnatal day. This sensitivity of immature muscles to succinylcholine is not related to detectable deficits in their functional innervation.

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Polymyxin B and Colistin: Activity, Resistance and Crossresistance *in vitro*.* (25511)

HANS A. HIRSCH,† CLARKE G. MCCARTHY AND MAXWELL FINLAND
(With technical assistance of Clare Wilcox and Joan H. Yarrows)
Thorndike Memorial Lab., Second and Fourth (Harvard) Medical Services, Boston City Hospital and Dept. of Medicine, Harvard Medical School, Mass.

Polymyxin B, a polypeptide antibiotic available for over a decade, has been used successfully in treatment of serious Gram-negative bacillary infections resistant to other antibacterial agents. It has been particularly effective against infections due to *Pseudomonas aeruginosa* but totally ineffective against Proteus infections; its use, however, has been limited by its neuro- and nephrotoxicity(1). Colistin, a new antibiotic recently introduced in Japan as Colimycin, is also a polypeptide and is produced by *Bacillus colistinus,* which is related to *B. polymyxa,* the source of polymyxin; it has similar antibacterial action but apparently lacks much of the toxic properties of polymyxin B. Both antibiotics have similar chemical constituents; each contains (+)-6-methyl-octanoic acid, d-leucine, l-leucine, l-threonine and 1-α,γ-diaminobutyric acid but polymyxin B contains d-phenylalanine which colistin lacks and the numbers of some of the other constituents differ(2-6). This paper deals with comparisons of *in vitro* activity of these 2 antibiotics against pathogenic organisms, most of them Gram-negative bacteria recently isolated from patients, and also reports observations on development of resistance and crossresistance to these 2 antibiotics *in vitro*.

Materials and methods. Polymyxin B sul-

* Aided by grant from Nat. Insts. of Health.
† Present address: Munich, Germany.

TABLE I. Susceptibility of Pathogenic Bacteria to Polymyxin B (P) and Colistin (C) *In Vitro.*

Organism	No. of strains*	Range of M.I.C., μg/ml P	Range of M.I.C., μg/ml C	Comparison of M.I.C.'s P = C %	P > C %	P > C Avg P/C	C > P %	C > P Avg C/P
Escherichia coli	26	.8- 3.1	.4- 3.1	42	23	1.8	35	1.6
Aerobacter	24†	.8->100	1.6->100	38	29	2.0	33	2.0
Klebsiella pneumoniae	13	.8- 100‡	.4- 100‡	38	38	1.5	23	1.8
Pseudomonas aeruginosa	29	3.1- 12.5	3.1- 12.5	17	0		83	1.8
Shigella	40	.8- 3.1	.4- 3.1	53	20	1.9	28	1.5
Salmonella	32	.8- 3.1	.8- 3.1	47	16	1.9	37	2.0
Neisseria gonorrhoeae	25	25 - 100	25 ->100	8	8	2.0	84	3.4
Hemophilus influenzae	43	.4- 3.1	.8- 25	5	5	1.6	91	4.0
Staphylococcus aureus	26	≥100§	>100					
All except *S. aureus*	232			30	15		55	

M.I.C. = Minimum inhibiting concentration, calculated as base.

* Only strains tested simultaneously with both P and C are included.

† 13 were *A. aerogenes* (M.I.C. 1.6-6.3) and 11 were *A. cloacae*; 3 of the latter were not inhibited by 100 μg/ml of P or C and 8 were susceptible to ≤25 μg/ml.

‡ Only 1 strain was inhibited by 100 μg/ml of P and C; the rest were inhibited by ≤6.3 and ≤3.1 μg/ml of P and C, respectively.

§ Only 1 strain was inhibited by 100 μg/ml.

fate (Aerosporin) was supplied by Burroughs Wellcome & Co.; colistin was supplied either as sulfate (Coly-mycin S) or as methanesulfonate (Coly-mycin M) by Warner Lambert Research Inst. but only the sulfate was used in the *in vitro* studies. All concentrations are expressed in terms of weight equivalent of base. Strains of most species tested for sensitivity to these antibiotics were recently isolated from blood, urine or infected exudates submitted for culture to the Bacteriology Laboratory, but most strains of *Escherichia coli* and *Klebsiella pneumoniae* were of specific types obtained from Dr. P. R. Edwards, Communicable Diseases Center, Chamblee, Ga., who also furnished strains of *Aerobacter cloacae and A. aerogenes*. Sensitivity of strains to the antibiotics was determined after growth for 24 hours on plates of heart infusion agar, Difco, pH 7.2±, into which 2-fold dilutions of the antibiotics were incorporated, the highest concentration tested being 100 μg/ml. "Chocolate" agar was used for strains of *Hemophilus influenzae* and *Neisseria gonorrhoeae,* and incubated under increased CO_2 (in candle jar) for 48 hours. Minimum inhibiting concentration (M.I.C.) was considered the lowest concentration with no perceptible growth or very slight growth visible only with a magnifying lens (3×); in comparing activity of the 2 antibiotics, the latter type of growth was arbitrarily rated as 50%, whereas moderate inhibition (+ growth, cf +++ growth on antibiotic-free medium) was rated as 75% of the 2-fold increment in M.I.C.

Results. Susceptibility of various organisms to Polymyxin B and Colistin. Results of tests for sensitivity of 258 strains of 9 genera or species of bacteria to the 2 antibiotics are summarized in Table I. All 26 strains of *S. aureus* were resistant to 100 μg/ml of both antibiotics, except one that was inhibited by this concentration of polymyxin. Several strains of various streptococci and other Gram-positive cocci as well as strains of Proteus (not listed in Table) were tested and found resistant to 100 μg/ml, except a strain of *S. lutea* which was inhibited by 50 μg/ml of polymyxin and 100 μg/ml of colistin. Of 232 strains of Gram-negative organisms listed in Table I, 30% were equally susceptible to both antibiotics, 55% were more susceptible to polymyxin and only 15% were more sensitive to colistin. The differences were usually only slight, and for many individual strains were less than one 2-fold dilution, *i.e.,* one antibiotic produced complete inhibition in the same minimum concentration that produced partial inhibition by the other. Average ratios of inhibiting concentrations are listed in Table I for strains of each genus or species in which the M.I.C. of the 2 drugs differed. Average ratio was usually less than 2, except for *H.*

TABLE II. Resistance and Crossresistance after Repeated Subcultures on Agar Containing Polymyxin B or Colistin.

	Antibiotic used for test	*Pseudomonas aeruginosa* (mucoid, green)	*Pseudomonas aeruginosa* (brown)	*Aerobacter aerogenes*	*Escherichia coli*
		μg/ml			
Parent strain:					
M.I.C.	P	12.5	3.1	1.6	1.6
	C	12.5	3.1	3.1	3.1
G.E.C.	P	3.1	.4	.4	.4
	C	3.1	.8	.8	1.6
After 30 subcultures on P:					
M.I.C.*	P	>100 (10)	6.3	>100 (5)	3.1
	C	" "	6.3	" (5)	3.1
G.E.C.*	P	" (20)	.8	" (8)	.8
	C	" "	.8	" (10)	.8
After 30 subcultures on C:					
M.I.C.*	P	" (10)	1.6	" (5)	1.6
	C	" "	6.3	" (5)	3.1
G.E.C.*	P	" (20)	.8	" (10)	.8
	C	" (17)	.8	" (6)	.8
After 30 subcultures on antibiotic-free agar:					
M.I.C.	P	3.1	6.3	1.6	1.6
	C	3.1	3.1	3.1	1.6
G.E.C.	P	.4	.4	.4	.4
	C	.8	.8	.8	.4

Abbreviations: P = polymyxin B; C = colistin; M.I.C. = minimum complete inhibiting concentration; G.E.C. = maximum concentration on which growth is equal to that of control culture on antibiotic-free agar.

* No. in parentheses indicates the No. of subcultures required to achieve this value (crossresistance tests done only after each fifth subculture on homologous antibiotic).

influenzae against which polymyxin was, on the average, 4 times as active as colistin for 91% of the strains, and among those of *N. gonorrhoeae,* for 84%, of which the M.I.C. of colistin was, on the average, 3.4 times greater than that of polymyxin.

Development of resistance and crossresistance in vitro. Two studies were carried out to produce resistant strains, one by multiple steps and the other by a single step. Four culturally and biochemically distinct strains originally susceptible to both polymyxin and colistin were used. In the first experiment, each strain was subcultured on 2 series of agar plates, one containing serial dilutions of polymyxin and the other similar dilutions of colistin, as in tests for sensitivity. After incubation for 48 hr at 37°C, 2 mm loopful of the growth was scraped from surface of plate from each series containing maximum concentration of antibiotic on which growth was equal to that on control plate of antibiotic-free agar (G.E.C.); this was suspended in 1 ml of broth and the suspension used to inoculate another similar series of plates containing the same antibiotic. After each 5 subcultures on the same antibiotic, the inoculum was also used simultaneously in test for sensitivity to the heterologous antibiotic. The M.I.C. and G.E.C. were recorded at each subculture and a total of 30 transfers on each antibiotic were thus carried out, the parent strains also being transferred on antibiotic-free agar each time. Changes in susceptibility to homologous and heterologous antibiotics are summarized in Table II. High degrees of resistance to each drug with complete crossresistance developed in the green strain of *Ps. aeruginosa* and in the strain of *A. aerogenes,* but the susceptibility of the brown Pseudomonas and the *E. coli* did not change significantly.

In the second experiment, the same 4 strains were used to derive single-step mutants by growing a large inoculum on the surface of antibiotic-containing agar. Sedimented organisms from 10 ml of a 24 hr growth in

TABLE III. Resistance and Crossresistance to Polymyxin B and Colistin in Single-Step Mutants.

Organism	Inoculum*	Antibiotic in agar†	No. of colonies At 48 hr	No. of colonies Tested	M.I.C., μg/ml Polymyxin B	M.I.C., μg/ml Colistin
Ps. aeruginosa (green)	10	P 100	0			
	10	C 100	5	2	12.5, 3.1	6.3, 12.5
	25	P 200	0			
	"	C 200	0			
	"	P 1000	0			
	"	C 1000	0			
Ps. aeruginosa (brown)	10	P 100	0			
	"	C 100	6	3	3.1, 6.3, 1.6	1.6, 1.6, .8
	25	P 200	5	2	3.1, 3.1	25, 25
	"	C 200	3	2	.4, .4	1.6, .8
	"	P 1000	1	1	no growth	no growth
	"	C 1000	2	2	"	"
A. aerogenes	10	P 100	>100	2	>100, >100	>100, >100
	"	C 100	?			
	25	P 200	5‡	2	" "	" "
	"	C 200	>100	2	" "	" "
	"	P 1000	> 50?	2	no growth	no growth
	"	C 1000	0			
E. coli	10	P 100	0			
	"	C 100	0			
	25	P 200	0			
	"	C 200	0			
	"	P 1000	0			
	"	C 1000	0			

* ml of culture, the centrifuged sediment of which was used as inoculum for each plate.
† P = polymyxin B, C = colistin; No. indicates concentration in μg/ml.
‡ Also >50 tiny colonies that failed to grow on subculture with or without antibiotic.

brain-heart infusion broth were resuspended in about 1 ml and flooded over the surface of 2 agar plates, one containing 100 μg/ml of polymyxin and the other a similar amount of colistin. The plates were allowed to stand with porous clay covers for several hours at room temperature until the surfaces were nearly dry, then incubated at 37°C with the porous covers until the surfaces were completely dry when glass covers were replaced and incubation was continued for 36 to 48 hr. After that time, the number of colonies that developed were noted and representative ones were picked, emulsified in small amount of broth and the suspension streaked on the surface of antibiotic-free agar and on agar containing 12.5 μg/ml of the homologous antibiotic to bring out any dependent variants. All organisms that grew on the antibiotic-containing agar also grew in absence of antibiotic, indicating that none of them were antibiotic-dependent. Growth from the antibiotic-free agar was then subcultured to broth and the resulting growth used to test for sensitivity to both antibiotics. The same procedure was repeated using the sediment of 25 ml of culture on agar containing 200 and 1000 μg/ml of each antibiotic.

The results are summarized in Table III. A few sensitive "persistors" were identified in growth of green Pseudomonas on 100 μg/ml of colistin and in growth of brown Pseudomonas in concentrations of 100 or 200 μg/ml of one or both antibiotics. One-step, highly resistant mutants, however, developed only in *A. aerogenes* on 100 or 200 μg/ml of either antibiotic. Dependent variants could not be demonstrated.

Comment. Our data indicate that colistin is comparable with polymyxin B in its activity *in vitro* against strains of organisms that are susceptible to the latter. Previous observations by Jawetz and Coleman(7) indicated that high degrees of resistance to polymyxin B could be obtained by daily subcultures using large inocula of *A. aerogenes* or *Ps. aeruginosa* that were originally of intermediate susceptibility to that antibiotic, but only a

slight rise in resistance could be achieved with 14 daily transfers of an originally highly susceptible strain of *E. coli*. Wright *et al.*(8) subjected 6 strains of *E. coli* to 20 subcultures in presence of polymyxin B and demonstrated increased resistance (12.5 to >400 μg/ml) in only one of them. Two of 4 strains showed marked increases in resistance by repeated subculture in either polymyxin or colistin and this was accompanied by complete cross-resistance to the heterologous drug. One of the same 2 organisms also yielded one-step highly resistant mutants, which also showed complete crossresistance.

Summary and conclusions. Polymyxin B and colistin, 2 closely related polypeptide antibiotics, had similar antibacterial activity against a large number of pathogenic bacteria. Polymyxin was somewhat more active than colistin against 55% of susceptible strains, on the average 3.4 and 4 times as active against most strains of *N. gonorrhoeae* and *H. influenzae*, respectively. Highly resistant variants developed on repeated subculture of a strain of *A. aerogenes* and one of *Ps. aeruginosa* on agar containing either of these antibiotics; complete crossresistance to the other antibiotic developed in each instance. Single-step mutants of the same strain of *A. aerogenes*, highly resistant to each antibiotic, were readily obtained by seeding a large inoculum on the surface of agar containing 100 or 200 μg/ml of that antibiotic; these mutants were completely cross-resistant to the heterologous antibiotic. Antibiotic-dependent variants were not encountered.

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Experimental Histoplasmosis in Monkeys.* (25512)

SAMUEL SASLAW, HAROLD N. CARLISLE AND JOANN SPARKS
Dept. of Medicine, The Ohio State University, Columbus

Previous studies from this laboratory have been concerned with pathogenesis of histoplasmosis in mice(1-4), dogs(5), and other large animals(6). The present report compares infectivity of *Histoplasma capsulatum* for *Macaca mulatta* when administered by intratracheal (IT), intranasal (IN), intragastric (IG) and intravenous (IV) routes.

Materials and methods. Young adult monkeys were inoculated by various routes above with 5 ml of saline suspension of yeast phase *H. capsulatum*† containing 2 ml of packed cells from 4-day cultures grown on brain heart infusion (Difco) blood agar. Eight monkeys were inoculated IT(7), 4 IN(8), 3 IG(9) and 2 IV (long saphenous). Blood cultures, taken 4, 7, 14, and 25 days after inoculation when possible, and all tissues taken at autopsy were treated and cultured by the method of Conant (10). The serologic response of monkeys was followed by collodion agglutination(11) and complement fixation(12) tests. Periorbital subcutaneous tests(13) for sensitivity to histoplasmin were carried out before inoculation and on surviving animals 6-7 weeks after inoculation.

Results. Table I summarizes the findings in 17 monkeys inoculated with *H. capsulatum* by various routes. All 8 monkeys inoculated IT exhibited clinical evidence of infection.

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† Strain G13—an isolate from spontaneous histoplasmosis in a dog, originally obtained from Charlotte Campbell Army Medical Center, Washington, D.C.