COMPARISON OF THE ACTION OF STREPTOMYCIN, POLYMYXIN B, AUREOMYCIN AND CHLOROMYCETIN ON *H. PERTUSSIS*, *H. PARAPERTUSSIS*, *H. INFLUENZAE* AND FIVE ENTERIC STRAINS OF GRAM-NEGATIVE BACILLI^{1, 2}

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Our recent investigations (1) suggest that knowledge of two factors operating *in vitro*—speed of lethal action on sensitive cells and frequency of resistant mutants—offers a reliable basis for predicting the therapeutic efficacy of streptomycin against eight different species of gram-negative bacilli. The data are summarized in Table I. The organisms responsible for infections which have been successfully treated with streptomycin—*H. influenzae*, *E. coli* and *H. pertussis*—have a low incidence of mutants which resist the action of 25 mcg. (micrograms) and 100 mcg. of streptomycin per c.c. In addition, their sensitive cells are killed rapidly by concentrations of streptomycin safely maintained in body fluids (10 mcg. per c.c.). On the other hand, in those infections in which streptomycin has not been found effective therapeutically a concentration of 10 mcg. per c.c. is not rapidly bactericidal for the sensitive cells, and the frequency of mutants resistant to 25 mcg. of streptomycin per c.c. is relatively high—10,000 to 100,000 times the rate of comparable mutants in successfully treated infections (Table I). In *Shigella* and *H. paraptussis* infections, in which streptomycin has not been clinically evaluated, one would predict success in the former and failure in the latter.

In an effort to improve therapeutic results in those infections in which streptomycin has failed therapeutically, either uniformly or as a result of emergence of resistance, three newer antibiotics—polymyxin B, aureomycin and chloromycetin—have been compared with streptomycin in their

action on the same eight species of gram-negative bacilli.

For this purpose three features of their activity have been studied:

1. The minimal concentrations of each agent needed to prevent growth completely over a 48-hour incubation period. A 24-hour period was used for aureomycin for most organisms.
2. The nature of their action on the eight species—primarily bacteriostatic or bactericidal—and the speed of any lethal action.
3. Predictable limitation of their therapeutic efficacy as a result of emergence of resistance and their influence on streptomycin-resistant mutants.

I. *Minimal effective concentrations*

A simple *in vitro* test, which provided valuable information of streptomycin action on these species, has been used to study the influence of polymyxin B, aureomycin and chloromycetin. This procedure determined the lowest concentration which completely prevented the growth of an inoculum of one to ten million organisms on an optimal agar medium after 48 hours' incubation. Because of the alleged lability of aureomycin *in vitro* the minimal effective concentration of this agent was assessed at 24 hours.

The results are seen in Table II. It is apparent that those organisms which require more than 25 mcg. of streptomycin per c.c. to prevent completely growth under the circumstances described are the causes of infections which have not been treated successfully with streptomycin. Polymyxin B appears to be the most efficient of the four agents and the broadest in its action. Aureomycin and chloromycetin are about equal in their range of action; the growth of each of the eight bacterial species is completely prevented by less than 25 mcg. per c.c. save for *Ps. aeruginosa*; the majority exhibited a high degree of sensitivity.

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TABLE I
Relation of Frequency of Streptomycin Resistant Mutants and
Speed of Lethal Action of Streptomycin on Sensitive Cells.

Species	Frequency of Mutants Resistant to Concentrations of Streptomycin			Speed of Lethal Action	
	25 mcg. per c.c.	100 mcg. per c.c.	1000 mcg. per c.c.	10 mcg. per c.c.	100 mcg. per c.c.
<i>H. influenzae</i>	10 ⁻¹¹	10 ⁻¹¹	10 ⁻¹¹	2-6 hrs.	10 min.
<i>H. pertussis</i>	10 ⁻¹⁰ to 10 ⁻¹¹	10 ⁻¹⁰ to 10 ⁻¹¹	10 ⁻¹⁰ to 10 ⁻¹¹	4-24 hrs.	2 hrs.
<i>E. coli</i>	10 ⁻¹⁰	10 ⁻¹⁰	10 ⁻¹⁰	6 hrs.	1 hr.
<i>Shigellae</i>	10 ⁻⁹ to 10 ⁻¹⁰	10 ⁻⁹ to 10 ⁻¹⁰	10 ⁻¹⁰	4 hrs.	1-2 hrs.
<i>Salmonellae</i>	10 ⁻⁶ to 10 ⁻⁷	10 ⁻⁹	10 ⁻¹⁰ to 10 ⁻¹¹	0*	2-4 hrs.
<i>S. typhosa</i>	10 ⁻⁵ to 10 ⁻⁶	10 ⁻⁸	10 ⁻¹⁰ to 10 ⁻¹¹	0	4-24 hrs.
<i>H. paraptussis</i>	10 ⁻⁶	10 ⁻⁸	10 ⁻¹⁰ to 10 ⁻¹¹	0	>24 hrs.
<i>Ps. aeruginosa</i>	10 ⁻⁵ to 10 ⁻⁶	10 ⁻⁷ to 10 ⁻⁸	10 ⁻¹⁰	0	4 hrs

* - Not primarily bactericidal

II. Speed of lethal action

Polymyxin B, aureomycin and chloromycetin have been compared with streptomycin in the speed of their lethal action on small populations (approximately 10,000 to 30,000 organisms per c.c.) in environments which will permit growth. After exposure to the antibiotics for two, six and

24 hours, samples of the broth cultures were examined for the number of viable organisms per c.c. by seeding into appropriate media in pour plate preparations after removal of any inhibitory concentration of the antibiotic, either through dilution or centrifuging and resuspending in normal broth. Table III presents the results on each of the eight species. The per cent of viable organisms remaining after exposure for six hours to two different concentrations of each of the antibiotics is listed.

It is apparent that streptomycin is primarily bactericidal in 10 mcg. per c.c. only against species for which it has been shown to be therapeutically effective. Polymyxin B exerts a rapid lethal action in concentrations of both 1 mcg. and 10 mcg. per c.c. Cultures of all species save for 0.07 per cent in *Salmonella* were sterile after two hours; shorter exposure times have not been studied. Chloromycetin in 10 mcg. per c.c. exerts a more rapid bactericidal action against *H. pertussis* and *H. paraptussis* than does aureomycin; against *H. influenzae* and *S. typhosa* the two agents are equal in the speed of their lethal action. Both aureomycin and chloromycetin are bacteriostatic for all of the other species with the exception of *Ps. aeruginosa*; in a concentration of 100 mcg. per c.c. aureomycin exerts a greater lethal action than does chloromycetin. On the other hand the

TABLE II

MINIMAL EFFECTIVE CONCENTRATIONS OF STREPTOMYCIN (SM), POLYMYXIN B (PMB), CHLOROMYCETIN (CM), AND AUREOMYCIN (AM) FOR 8 SPECIES OF GRAM NEGATIVE BACILLI				
SPECIES	ANTIBIOTIC -mcg/cc.			
	SM	PMB	CM	AM*
<i>H. INFLUENZAE</i>	1-3	0.3	1	0.5-1
<i>H. PERTUSSIS</i>	3	1-5	1	3
<i>E. COLI</i>	75-25	0.5-3	5-<25	5-<25
<i>SHIGELLAE</i>	25	1	3-10	3-5
<i>SALMONELLAE</i>	.25-200	1-5	5	5-<25
<i>S. TYPHOSA</i>	25-75	0.5	3-5	3
<i>Ps. AERUGINOSA</i>	100-200	1-3	>100	50
<i>H. PARAPTUSSIS</i>	>25	5	3	5->10
* 24 hour reading except for <i>H. pertussis</i> and <i>H. paraptussis</i> .				

results of exposure for 24 hours, not listed here, show that stasis of growth by chloromycetin persists through 24 hours in cultures of all of these organisms save for *Ps. aeruginosa*, and the number of viable cells even decreases significantly, whereas the static effect of aureomycin is seen after 24 hours only against *Shigella* and *S. typhosa*; these results may only reflect the deterioration of aureomycin. However the persistence of bacteriostasis with some organisms suggests that other factors are playing a role.

Ps. aeruginosa is not even inhibited by a six-hour exposure to streptomycin, chloromycetin or aureomycin in a concentration of 10 mcg. per c.c., whereas polymyxin B in a concentration of 1 mcg. per c.c. is bactericidal within two hours.

The greater bactericidal action of 100 mcg. per c.c. of streptomycin is worthy of emphasis since in infections of the meninges and urinary tract a comparable concentration can be maintained in these fluids without damage in most patients.

III. Resistance problem

The study of the limitation of therapeutic efficacy of these agents by the phenomenon of resistance was approached in two ways: 1) examination of large populations for presence of cells resistant to these antibiotics, and 2) examination of

TABLE IV

MINIMAL EFFECTIVE CONCENTRATIONS OF POLYMYXIN-B (PMB), CHLOROMYCETIN (CM), AND AUREOMYCIN (AM) FOR STREPTOMYCIN (SM) RESISTANT MUTANTS OF 8 SPECIES.				
SPECIES	ANTIBIOTIC - mcg./cc.			
	SM	PMB	CM	AM
H. INFLUENZAE	>1000	0.1	1	0.5
H. PERTUSSIS	>1000	5	1	0.5
E. COLI	>1000	0.3-3	5-25	3-5
SHIGELLAE	>1000	0.5	1	3-5
SALMONELLAE	>1000	1-3	5	3-5
S. TYPHOSA	>1000	0.3-0.5	3	3
PS. AERUGINOSA	>100	1-3	25-100	3-25
H. PARAPERTUSSIS	>1000	5-25	1-3	1-3

B. SIMANOW

their action on streptomycin-resistant mutants of each of these species.

It has already been reported (2-4) that mutants resistant to 1000 mcg. of streptomycin per c.c. may be easily demonstrated in large populations of each of the eight species; mutants resistant to 25 mcg. and 100 mcg. of streptomycin per c.c. are uniformly present in smaller populations of the species against which this antibiotic has not been effective therapeutically (1).

Large populations, 60 to 600 billions, of each of the eight species have been examined for presence of cells resistant to polymyxin B, 10 mcg. and 100 mcg. per c.c. and chloromycetin 10 mcg. per c.c.; the supply has not permitted study of a higher concentration. *H. pertussis* and *H. parapertussis* have been examined for cells resistant to 10 mcg. and 100 mcg. of aureomycin per c.c.

It was not possible to demonstrate cells resistant to 100 mcg. per c.c. of polymyxin B in any species by this method, and only in *Salmonella* and *Ps. aeruginosa* was it possible to demonstrate a minute fraction of cells resistant to 10 mcg. per c.c.; when a few of the colonies of these two species which formed in agar containing 10 mcg. per c.c. were tested for their sensitivity to this concentration only an occasional colony grew in polymyxin B agar (10 mcg. per c.c.) but growth was normal on control medium.

TABLE III

PER CENT VIABLE CELLS AFTER 6 HRS. EXPOSURE TO STREPTOMYCIN (SM), POLYMYXIN B (PMB), CHLOROMYCETIN (CM), AND AUREOMYCIN (AM).								
SPECIES	SM		PMB		CM		AM	
	mcg./cc.		mcg./cc.		mcg./cc.		mcg./cc.	
	10	100	1	10	10	100	10	100
H. INFLUENZAE	0	0	0	0	0	0	0.96	0
H. PERTUSSIS	0.03	0		0	0.1 2.0	0.03 3.0	10.	0.1
E. COLI	0	0	0	0	100	52 65	128	1.8
SHIGELLAE	0	0	0	0	100	65 79	100	20
SALMONELLAE	1.4 100	0	0.01	0	100	26 35	100	8.6
S. TYPHOSA	37 633	0.4	0	0	54 60	12 22	74	0.2
PS. AERUGINOSA	287 1500	0	0	0	10,100	15	170	2.4
H. PARAPERTUSSIS	100	10 25		0	0.4 0.5	1.0	2. 23	0 0.1

B. SIMANOW

While a few colonies grew when large populations of *E. coli*, *Salmonella* and *S. typhosa* were seeded in pour plate preparations of agar containing 10 mcg. per c.c. of chloromycetin, none of them on subculture grew on chloromycetin agar (10 mcg. per c.c.).

The investigation of resistance of these newer agents is still in progress but it is already apparent that resistance to polymyxin B and chloromycetin differs from streptomycin resistance; the data suggest that emergence of resistance will play a less important role in therapy.

The influence of polymyxin B, chloromycetin and aureomycin on cells highly resistant to streptomycin is shown in Table IV for each of the eight species. The sensitivity of these streptomycin-resistant strains does not differ significantly from those strains which are sensitive to streptomycin. Therefore it is apparent that the mechanism of action of each of these agents differs from the mode of action of streptomycin.

These data suggest a number of clinical applications. Polymyxin B would be the treatment of choice for all of these infections, but its toxic action precludes its clinical use unless the injurious substance can be removed without altering the antibacterial action. A final assessment of aureomycin *in vitro* must await additional factual data. Chloromycetin because of absence of toxic manifestations, its rapid lethal action on *H. influenzae* and *H. pertussis* and the rarity of resistant mutants warrants a clinical trial in these infections. In *E. coli* and *Shigella* infections there

is reason to anticipate a more rapid elimination of the organisms by the combined action of sulfadiazine and streptomycin than by aureomycin or chloromycetin alone. In *Salmonella* and *S. typhosa* infections, either chloromycetin or aureomycin should be a more effective agent than streptomycin. For *Ps. aeruginosa* infections we can expect little therapeutic value from streptomycin, aureomycin or chloromycetin used as a single agent if concentrations in the body fluids do not exceed 10 mcg. per c.c. The results suggest that the combined action of streptomycin and aureomycin may prove of therapeutic value in *Ps. aeruginosa* infections of body fluids in which concentrations of 50 mcg. to 100 mcg. per c.c. can be maintained with safety. In *H. paraptussis* infections chloromycetin should prove to be the treatment of choice.

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