Cross-Resistance Among 3 Tetracyclines.* (20778)

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In a previous paper(1) it was shown that the 3 chemically closely related antibiotics tetracycline, oxytetracycline (Terramycin) and chlortetracycline (Aureomycin) exerted very similar antibacterial action against most of the pathogenic strains which were studied. In general, the bacteria tested were equally sensitive or resistant to all 3 analogues under the conditions of the test that was used, although for individual strains or species there was a significant quantitative difference in the action of one or another of these analogues. Earlier studies(2-7) had shown considerable cross-resistance between chlortetracycline and oxytetracycline and some cross-resistance between these 2 agents and chloramphenicol. It was, therefore, of interest to determine whether similar cross-resistance could be demonstrated *in vitro* with the new antibiotic, tetracycline, which lacks both the organic chlorine ion of chlortetracycline and the OH group which characterizes oxytetracycline.

Materials and methods. Tetracycline (Achromycin) hydrochloride was provided in crystalline form in sterile vials by Dr. Stanton M. Hardy of Lederle Laboratories. The other antibiotics employed were similar to those used in previous studies(8). The organisms used were recently isolated from single colony cultures of infectious material from patients in the hospital. These were grown in brain-heart infusion broth (Difco), pH ± 7.4, containing serial 4-fold dilutions of tetracycline and of oxytetracycline, and subcultures were made at 3- or 4-day intervals from the tube containing the highest concentration of antibiotic which permitted nearly optimum growth to another series of tubes of broth containing the same antibiotic. (Chlortetracycline was not used for such subcultures because during such long periods of incubation deterioration of that antibiotic is so great as to render the results invalid for comparisons.) After 20 such subcultures, the 2 series of strains from the antibiotics and their parent strains which had not been exposed to any antibiotics, were tested simultaneously to each of the 3 tetracycline analogues by a 2-fold agar plate dilution method(9). All of the organisms were then subcultured once in antibiotic-free broth and strains from all 3 series were tested simultaneously for sensitivity to penicillin, streptomycin, chloramphenicol, bacitracin, polymyxin B (Aerosporin), neomycin and erythromycin by the same agar plate dilution method. The sensitivity of any strain was considered to be the minimum concentration of the antibiotic which failed to produce visible growth in 24 hours.

Results. Cross-resistance among the tetracyclines. The sensitivities to tetracycline, oxytetracycline, and chlortetracycline of the parent strains and of those which had been subcultured 20 times in broth containing graded concentrations of either tetracycline or oxytetracycline are listed in Table I. Each of the organisms increased in resistance from 16- to 256-fold against the antibiotic to which it had been exposed. All of these organisms also increased in resistance against each of the other 2 analogues, and the increases were of the same order of magnitude as against the homologous antibiotics; in some instances the increases in resistance against the heterologous agent exceeded those which were demonstrated against the antibiotic to which the organisms had been exposed, but the reverse was observed just as frequently.

Cross-resistance to other antibiotics. Tests with the 3 series of cultures showed no significant differences in sensitivity to penicillin, streptomycin, bacitracin, polymyxin B, neomycin or erythromycin; in every instance the minimum inhibiting concentration of the tetracycline-resistant and oxytetracycline-resistant variants for each of these antibiotics was

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TABLE I. Cross Resistance to Other Tetracycline Analogues after Repeated Subcultures in Broth Containing Tetracycline or Oxytetracycline.

Strain	Minimum inhibiting concentration, μg/ml								
	Tetracycline			Oxytetracycline			Chlortetracycline		
	P*	T×20	O×20	P	T×20	O×20	P	T×20	O×20
K. pneumoniae	6.3	800	800	6.3	800	800	25	400	400
Enterococcus 1	1.6	50	800	3.1	100	800	3.1	50	100
" 2	1.6	50	800	3.1	100	800	3.1	50	100
Staph. aureus 1	.8	25	100	.8	25	50	1.6	25	25
" " 2	.8	100	25	1.6	200	50	1.6	50	12.5
E. coli	3.1	400	50	3.1	200	50	12.5	200	100
A. aerogenes	6.3	800	800	6.3	800	800	12.5	200	200
Strep. viridans	1.6	100	50	.8	100	200	1.6	50	50

* P = parent strain not previously exposed to antibiotics; T×20 = same strain after 20 subcultures in broth containing tetracycline; O×20 = same strain after 20 subcultures in broth containing oxytetracycline.

TABLE II. Cross-Resistance to Chloramphenicol in Organisms Exposed to Tetracycline or Oxytetracycline.

Organism	M.I.C.* of chloramphenicol, μg/ml		
	Parent strain	Strain subcultured 20 times in Tetracycline	Strain subcultured 20 times in Oxytetracycline
K. pneumoniae	50	>400	>400
Enterococcus 1	25	50	25
" 2	25	25	25
Staph. aureus 1	25	50	25
" " 2	25	100	25
E. coli	25	400	400
A. aerogenes	100	>400	>400
Strep. viridans	6.3	25	25

* Minimum inhibiting concentration.

either identical with that of the parent strain or varied from it by only a single 2-fold dilution. The sensitivities to chloramphenicol, however, showed significant changes and are listed in Table II. As in the previous study with chlortetracycline and oxytetracycline(7), only the coliform organisms which had been made resistant to the tetracyclines exhibited significant cross-resistance to chloramphenicol, whereas the tetracycline- and oxytetracycline-resistant gram-positive organisms showed either no cross-resistance or only slight increases in resistance to chloramphenicol.

Miscellaneous observations. The strains of the 20th transfer in the antibiotics were also compared in several ways with their respective parent strains. On antibiotic-free blood agar plates, the strains transferred directly from the antibiotic containing broth grew more slowly and formed much smaller colonies. This was particularly striking with the staphylococci of which there were tiny colonies, visible only after magnification, interspersed among somewhat larger colonies; the small ones corresponded morphologically to the so-called G forms(10). These staphylococcal colonies also produced less pigment than the parent strain. The strain of *Staph. aureus* 2 which had been subcultured in oxytetracycline failed to coagulate human plasma, whereas all of the other staphylococcal strains did so when tested at the same time with the same plasma. Penicillinase was produced by both the parent and the resistant variants of *Staph. aureus* 2, when the latter were tested after a single subculture in antibiotic-free broth. On eosin-methylene blue agar, the 3 coliform organisms that had been subcultured in the antibiotics produced smaller colonies than their respective parent strains, and the colonies of the antibiotic-resistant variants of *K. pneumoniae* were flatter and less mucoid than those of their parent strain. On gram stain the organisms taken directly from the antibiotic-containing broth were generally smaller and much more pleomorphic than their respective parent cultures grown in the same broth without antibiotic. The resistant gram-positive organisms stained poorly and the resistant variants of *K. pneumoniae* had lost their capsules. Resistance to heat was retained by all the strains of enterococci. The reaction of *E. coli* and *A. aerogenes* to Simmons citrate agar was unchanged.

Conclusions. Organisms made resistant *in vitro* to either tetracycline or oxytetracycline by repeated subcultures in these antibiotics

developed essentially complete cross-resistance to each other and to chlortetracycline. No cross-resistance or increases in sensitivity to penicillin, streptomycin, bacitracin, polymyxin, neomycin or erythromycin developed as a result of the increases in resistance to the tetracyclines. Three strains of coliform bacilli each showed significant cross-resistance to chloramphenicol following subcultures in either tetracycline or oxytetracycline, but similar cross-resistance was not observed in 5 gram-positive coccal strains.

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Prevention of Urinary Calculi Formation in Mink by Alteration of Urinary pH.* (20779)

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Chemical analyses of mink urinary calculi (1) have shown that the main component is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$). Small amounts of calcium phosphate and magnesium phosphate may also be present. Magnesium ammonium phosphate calculi are a type of calculi known to form in an alkaline urine. *In vitro* studies(2) have shown that magnesium ammonium phosphate is quite soluble in water when the pH is reduced to 6.0 or less. However, there is a sharp drop in the solubility of this compound when the pH range is 6.0 to 7.5. The pH of urine from mink on ranch diets varies from 5.5 to 7.5(3,4). It appears that the pH of the urine is an important factor in the formation of urinary calculi in mink.

The alteration of urinary pH by dietary means may be a logical method to attack the urinary calculi disease problem in mink. A practical and inexpensive means of acidifying the urine is the addition of the chemical ammonium chloride (NH_4Cl) to the diet. Studies on the use of ammonium chloride for the prevention of urinary calculi in mink are presented in this report.

Experimental. Mink were placed in individual metabolism cages and were given the following ranch diet: commercial mink cereal,† 25%; horsemeat (with bone), 60%; and horse liver, 15%. During the ammonium chloride feeding period (Experimental Period

TABLE I. Alteration of Mink Urinary pH by Ammonium Chloride.

Exp. period	Diet	Urinary pH* Avg	Range
I	Ranch diet	6.4	6.0–6.8
II	Ranch diet + 1 g NH_4Cl/mink/day	5.7	5.5–5.9

* Avg of 5 different 24-hr urine collections from 17 mink.

† Federal Xtra Mink Mix.

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