Antibacterial Action of Tetracycline: Comparisons with Oxytetracycline and Chlortetracycline.*† (20774)

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The importance of minor changes in chemical structure in affecting the antimicrobial action as well as the pharmacologic and toxic properties of substances is well recognized in the field of chemotherapy. The preparation of tetracycline(1,2) by the catalytic hydrogenation of chlortetracycline (Aureomycin) offered an opportunity to compare the properties of 3 closely related antibiotics, each varying from the other by a single atom. Tetracycline has a structure common to both chlortetracycline and to oxytetracycline (Terramycin), lacking both the chlorine atom which is present on the aromatic ring of chlortetracycline(3) and absent from oxytetracycline (4) and an OH group which distinguishes oxytetracycline but which is also absent from chlortetracycline. Comparisons of the antibacterial action of these three tetracycline analogues are presented in this paper.

Materials and methods. Tetracycline (supplied in the latter part of this study under the trademark Achromycin) and chlortetracycline (Aureomycin) were furnished by Lederle Laboratories through the courtesy of Dr. Stanton M. Hardy. Oxytetracycline (Terramycin) was provided by Chas. Pfizer & Co. through the kindness of Dr. Gladys L. Hobby. These three antibiotics were each made available as crystals of the hydrochloride in sterile, rubber-capped vials containing 100 or 500 mg equivalent of the base. The strains of bacteria studied were from various infected materials obtained from patients in the hospital; most of them were isolated and identified in the bacteriological laboratory of the Mallory Institute of Pathology by Miss Marion E. Lamb and Miss A. Kathleen Daly. The strains of *Neisseria gonorrhoeae* were isolated by Mrs. Helen Trousdale of the Genitourinary Clinic, and most of the strains of *Neisseria meningitidis* were furnished by Dr. Sara E. Branham of the National Microbiological Institute. Tests for sensitivity were carried out by streaking a loopful of freshly grown broth culture onto the surface of each of a series of plates of heart infusion agar (Difco pH ± 7.4) containing 2-fold dilutions of each of the antibiotics. Visible growth was recorded after 24 hours and again after 48 hours of incubation at 37°C, except in the case of Neisseria which were read only after 48 hours. Horse blood was incorporated in the agar for the pneumococci, streptococci, staphylococci and Neisseria. The sensitivity of a strain was considered to be the minimum concentration of antibiotic which produced marked inhibition of growth at 24 hours. In making comparisons, however, the complete inhibition (no detectable growth) after 48 hours was also used. Growth curves were carried out with cultures of *Bacillus cereus* No. 5 (obtained from Dr. A. C. Dornbush of the Lederle Laboratories) and *Streptococcus 98* in brain heart infusion broth (Difco pH ± 7.4), with and without various concentrations of the three antibiotics, and determining the number of viable organisms at appropriate intervals in pour plates of the cultures in antibiotic-free agar. The seed cultures had been grown overnight in broth and horse blood was incorporated in all cultures of *Streptococcus 98*. Assays for each of the 3 tetracyclines in serum and urine, and also for standard solutions of these antibiotics were carried out with *B. cereus* No. 5 by a serial two-fold dilution method in broth.

Results. Effect of storage in the cold. Citrated plasma from venous blood, in amounts

* Summary of some of these findings presented at the Antibiotic Symposium, Washington, D.C., Oct. 28, 1953.

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TABLE I. Sensitivity of 257 Recently Isolated Strains of Common Pathogenic Bacteria to 3 Tetracyclines.

Organism	No. of strains tested	Anti-biotic*	Minimum inhibiting concentration (M.I.C.), μg/ml														M.I.C. for ⅔ of strains
			>200	200	100	50	25	12.5	6.3	3.1	1.6	.8	.4	.2	.1	.04	.02	
D. pneumoniae	22	T										1	1	14	6			.2
		O											1	9	10	1		.2
		C										1	2	10	4	4	1	.2
Strep. viridans	15	T										1	8	3	2	1		.4
		O										1	8	2	2	1	1	.4
		C										1	0	10	3	0	1	.2
Strep. hemolyticus	24	T				2	0	1	5	7	5	4						6.3
		O				1	1	0	9	5	5	3						6.3
		C				2	0	9	4	2	6	1						12.5
Enterococcus	14	T	8	0	0	0	0	0	5	1								>200
		O	6	1	1	0	0	1	4	1								>200
		C	2	1	3	2	0	0	1	0	5							100
Staph. aureus	17	T	6	0	0	0	0	0	0	1	4	5	1					3.1
		O	6	0	0	0	0	0	0	0	7	3	1					3.1
		C	3	2	0	1	0	0	0	0	1	10						1.6
N. gonorrhoeae†	38	T								2	4	7	15	10				.8
		O									3	6	23	6				.4
		C								8	10	14	3	3				1.6
N. meningitidis†	24	T								6	12	6						1.6
		O								5	11	8						1.6
		C					1	3	5	15								6.3
E. coli	28	T							12	8	8							6.3
		O							10	8	7	3						6.3
		C					4	7	17									12.5
A. aerogenes	21	T	1	0	2	3	4	2	3	3	2	1						25
		O	1	0	1	3	7	1	0	3	3	2						25
		C	1	0	3	5	1	2	2	4	1	2						50
Shigella sonnei	14	T						7	5	2								12.5
		O						7	7									12.5
		C					7	0	7									25
B. proteus	6	T	2	2	1	1												200
		O	6															>200
		C	5	1														>200
P. aeruginosa	34	T	4	9	10	5	2	1	0	0	0	3						200
		O	2	3	18	3	1	1	1	1	1	3						100
		C	14	6	5	1	1	4	0	1	1	1						>200

* T = tetracycline; O = oxytetracycline; C = chlortetracycline.

† Tests of these organisms were read after incubation for 48 hr; all others were read at 24 hr.

sufficient for several replicate assays, was obtained from 6 patients after oral or intravenous administration of tetracycline. Similar samples of plasma were also obtained at several intervals following ingestion of 0.5 g of one of the 3 tetracyclines given to 6 subjects, each antibiotic being given to 2 of them. Samples of urine were also obtained from the same subjects. Assays for the antibiotic content of the plasma and urine were carried out with these samples promptly and the assays were repeated after they had been stored in the frozen state at –25°C for varying periods up to 3 weeks. No deterioration in antibiotic activity of these specimens could be determined by this test. Standard solutions of 2 μg/ml of each of the 3 antibiotics were also prepared in broth; these were tested promptly and again at intervals up to 3 weeks of storage at 5° to 10°C (in a commercial refrigerator) and at –25°C (in a commercial "deep-freeze"). The solutions all retained their activity essentially unimpaired during the 3 weeks of storage at –25°C. At 5° to 10°C the chlortetracycline solution lost about 50% of its activity (difference of one dilution tube) between the second and third day, and 75% of its activity was lost (a difference of 2 dilution tubes) between the 4th and 7th day; the oxytetracycline lost 50% of its activity between the 4th and 7th day but the tetracycline solution showed no loss of activity as demonstrated by this test during 3 weeks of storage.

Sensitivity of recently isolated strains. The results of tests for sensitivity of 257 strains of common pathogens carried out simultaneously with each of the 3 tetracycline analogues are shown in Table I. On the whole, the strains of any given species or genus varied in their sensitivity to the 3 tetracyclines over a similar range of concentrations, and the minimum concentration required to inhibit two-thirds of the strains of each species or genus that was tested likewise did not differ widely except in the case of Neisseria for which the minimum inhibiting concentration of chlortetracycline was greater than for the other 2

TABLE II. Comparison of Sensitivities of Individual Strains to 3 Tetracyclines.

Organisms	No. of strains tested	Hr of incubation*	M.I.C. oxytetracycline — M.I.C. tetracycline							M.I.C. chlortetracycline — M.I.C. tetracycline						
			8	4	2	1	½	¼	⅛	8	4	2	1	½	¼	⅛
D. pneumoniae	22	24				16	5	1				3	11	6	1	1
		48				11	10	1				3	14	4	1	
Strep. viridans	15	24			1	10	4						6	7	1	1
		48			1	9	5				4	4	6	1		
Strep. hemolyticus	24	24		3	4	11	5	1		3	4	8	5	3	1	
		48		4	5	6	5	3	1	5	3	6	9	1		
Enterococcus	14	24			1	11	0	1	1			1	3	5	3	2
		48			1	11	1	1			6	0	6	2		
Staph. aureus	17	24			3	12	2					1	8	7	0	1
		48			5	12				9	2	0	5	1		
N. gonorrhaeae	38	48			8	19	6	5		2	16	13	5	1	1	
N. meningitidis	24	48		1	3	12	8			4	10	5	5			
E. coli	28	24	2	2	6	7	3	8		7	6	9	6			
		48		6	4	6	9	3		14	9	5				
A. aerogenes	21	24		1	1	12	7				1	5	11	3	1	
		48			4	10	6	1			9	7	5			
Shigella sonnei	14	24			2	12						9	5			
		48			7	7					7	7				
B. proteus	6	24	4	2						1	1	1	3			
		48	4	2						2	1	3				
P. aeruginosa	34	24		1	3	13	11	4	2		9	9	12	2	2	
		48		1	0	9	22	2			4	6	22	2		
All strains	195	24	6	9	21	104	37	15	3	11	21	46	70	33	9	5
	257	48	4	14	38	112	72	16	1	36	71	59	77	12	2	

* Endpoints read as marked inhibition at 24 hr, complete inhibition at 48 hr.

agents. The differences in sensitivity of the individual strains to the 3 analogues are summarized in Table II. About one-half of all the strains tested were equally sensitive to tetracycline and oxytetracycline and the remainder were distributed in a normal manner between those more sensitive and those more resistant; this was true both when the tests were read at 24 hours and again when they were read at 48 hours. The comparisons of the tests with tetracycline and those with chlortetracycline, however, reflected the deterioration of the latter during the course of the tests. This was manifested in the 24-hour readings by the large number of strains which required from 2 to 8 times as much chlortetracycline as compared with tetracycline to inhibit. This phenomenon was further exaggerated when the tests were read after 48 hours, at which time about 60% of the strains required the larger concentrations of chlortetracycline. In a number of instances, however, one of the analogues showed greater activity than the others against an appreciable proportion of strains of some species or genus. Thus, oxytetracycline appeared to be the most active against a large proportion of the strains of Pseudomonas; tetracycline appeared to be the most active against the few strains of Proteus that were tested, although high concentrations of all of the antibiotics were required to inhibit these strains. In the tests read at 24 hours chlortetracycline appeared to be the most active against a large proportion of the strains of Enterococcus and *Staphylococcus aureus*. The larger amounts of chortetracycline required to inhibit the strains of *Neisseria gonorrhoeae* and *N. meningitidis*, however, may be considered as reflecting the deterioration of that antibiotic in the course of the test. However, chlortetracycline appeared to be the least active against most of the strains of *E. coli* and *Shigella sonnei* even when the tests were read at 24 hours.

Growth curves. The effects of various concentrations of each of the 3 tetracyclines on the growth of *B. cereus* No. 5 and of *Streptococcus 98* are depicted in Fig. 1 and 2, respectively. The same inoculum (about 10^4 organisms/ml) was used in each instance and corresponded to that generally employed in

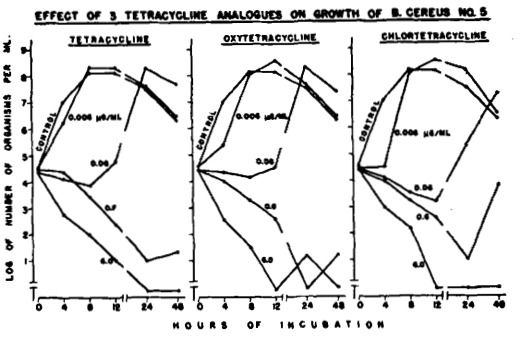

FIG. 1.

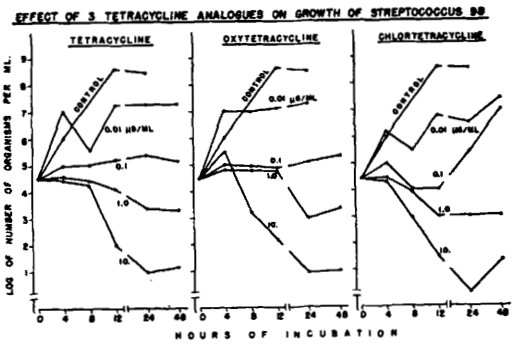

FIG. 2.

this laboratory for antibiotic assays and in tests for sensitivity which employs serial dilutions of antibiotics in broth. The growth curves for each of the 3 antibiotics are very similar in the case of both organisms, the major difference being the earlier resumption of growth following inhibition in the presence of chlortetracycline in some instances. The effect of the size of the inoculum is shown for *B. cereus* in Fig. 3; the inocula used for the growth curves in the upper panels of this figure were 100 times greater than those shown in Fig. 1 and the inocula for the curves in the lower panel were such as to provide 30 to 60 organisms per ml. The tests with each inoculum and the 3 antibiotics were done at the same time, but those with the different inocula were done at different times. Tetracycline appeared to exert its inhibitory action for a longer period than did the other 2 analogues, but other differences among the antibiotics were relatively minor.

Discussion. The data presented indicate that, for the most part, the antibacterial action of each of the 3 tetracycline analogues is

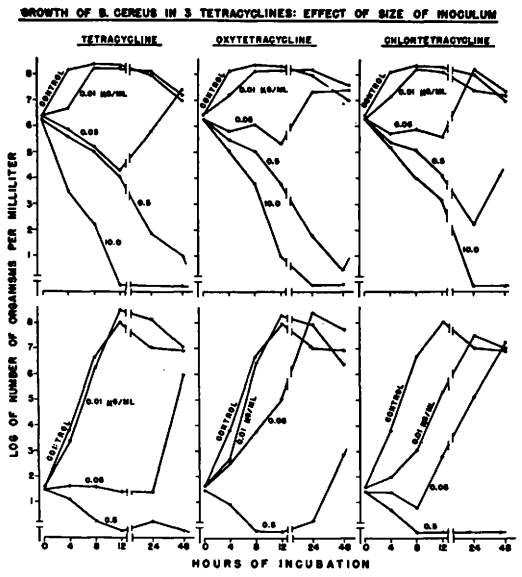

FIG. 3.

very similar under the conditions employed. In the case of individual strains, however, there were significant quantitative differences. The instability of chlortetracycline in the usual *in vitro* tests is well known. The effect of pH was studied and further differences were noted; these have varied somewhat when different organisms were used and the results are not presented since they require further study. In general all 3 agents were more active in an acid medium; this was particularly true of chlortetracycline which was the least active in an alkaline range, at which it was also the most unstable.

In the present study, recently isolated strains were used. This is important because of the increasing incidence of strains of certain organisms which are resistant to both oxytetracycline and chlortetracycline, presumably as a result of the wide use of these agents(5). In the tests for sensitivity, strains resistant to one of the tetracyclines were usually resistant in about the same general range to the other 2 analogues. In a separate study(6), a group of organisms made resistant *in vitro* by repeated subcultures in increasing concentrations of tetracycline and of oxytetracycline were subsequently tested and found to have acquired resistance to all 3 analogues to about the same degree.

Conclusions. The antibacterial action of tetracycline, oxytetracycline and chlortetracycline *in vitro* appeared to be very similar although significant quantitative differences are noted in the action of these 3 analogues on individual strains and under certain cultural conditions.

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Effect of Total Body X-Irradiation on the Tryptophan Peroxidase Activity of Rat Liver. (20775)

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Knox has shown that the activity of tryptophan peroxidase-oxidase (TPO) of rat liver can be augmented *in vivo* by administration of large doses of the substrate, tryptophan, and to a lesser extent by injection of certain other amino acids, epinephrine, or histamine (1). The increase after tryptophan was considered to be an adaptive response, while the effect of non-specific compounds was attributed to pituitary-adrenal stimulation, since adrenalectomy inhibited the response to non-substrate compounds but not to tryptophan.

It was of interest to study the effects of total body x-irradiation on TPO of rat liver