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Blood Levels and Urinary Excretion in Normal Subjects after Ingestion of Tetracycline Analogues.*† (20785)

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The antibacterial action of the 3 chemically closely related antibiotics—tetracycline, oxytetracycline (Terramycin), and chlortetracycline (Aureomycin)—was found to be very similar against most of the strains of common human pathogenic bacteria that were tested (1). The results of assays of blood and urine for antibiotic activity following oral administration of these 3 agents are presented in this paper.

Materials and methods. Tetracycline (Achromycin) and chlortetracycline (Aureomycin) were supplied by Dr. Stanton M. Hardy of the Lederle Laboratories, and oxytetracycline (Terramycin) was provided by Dr. Gladys L. Hobby of Chas. Pfizer & Co. Each of these antibiotics was furnished in both capsules and tablets containing 250 mg equivalent of the free base; all were in the form of the hydrochlorides, except the oxytetracycline tablets, which were of the free base. The subjects were male members of the hospital staff who had not received any antibiotics for several weeks prior to the beginning of the study. Assays for antibiotic activity were done on citrated plasma from venous blood and on "clean" voided specimens of urine; 2-fold dilutions were made in brain-heart infusion broth (Difco pH ± 7.4) and seeded with an equal volume of a 10^{-4} dilution of an overnight growth of *B. cereus* No. 5 (obtained from Dr. A. C. Dornbush of Lederle Laboratories). The tests with plasma were read for visible growth after incubation at 37°C for 18 hours (and in some instances they were examined again after 24 and 40 hours); assays of urine were observed at 8, 18, and 24 hours. The results were recorded as the maximum final dilution completely inhibiting growth, as indicated by absence of visible clouding of the medium. The minimum inhibiting concentration in the specimens was determined by comparison with freshly prepared standard solutions assayed simultaneously and read at 18 hours.

Results. Two separate experiments were carried out. In the first, 12 subjects were given 1.0 g of each of the 3 analogs in rotation 4 to 7 days apart, 2 receiving capsules and 2, tablets of one of the 3 tetracyclines on each of the 3 days. None of the participants knew which of the analogs were given until the entire experiment was completed. Plasma and urine were collected at specified intervals, and those obtained at each interval were assayed simultaneously so that samples from all the subjects who had taken each of the 3 antibiotics were included in each test. The levels of antibiotic in the plasmas are shown in Fig. 1. None of those obtained before the doses showed inhibition of the test organism. More than one-half of the subjects had no demonstrable level at one hour, but the levels increased to a maximum between 2 and 6 hours. Two subjects who received tablets of oxytetracycline base showed no antibiotic

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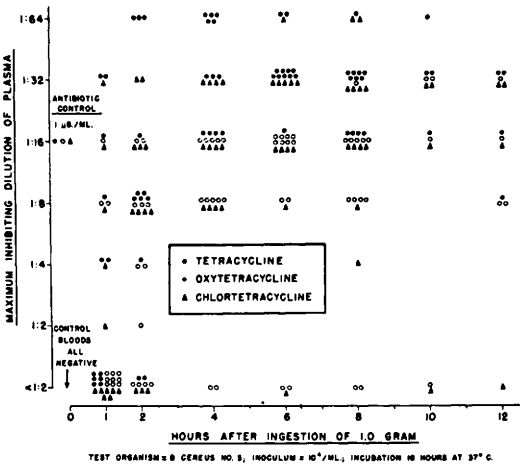

FIG. 1.

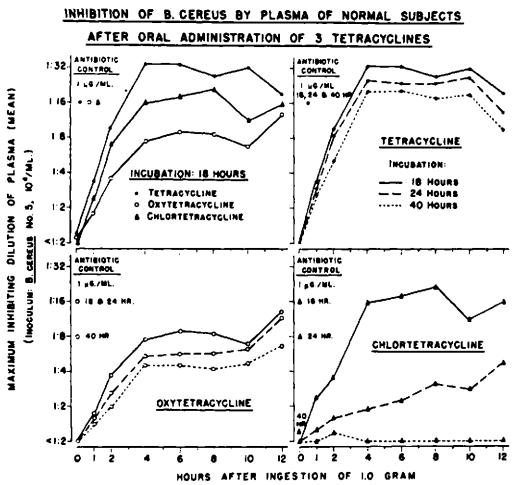

FIG. 2.

activity at any time. The levels for each of the antibiotics, as judged from the tests read at 18 hours, were quite similar, but they appeared to be highest after tetracycline and lowest after oxytetracycline. This is best shown in the average level as depicted in the upper left panel in Fig. 2. The effect of the rapid deterioration of chlortetracycline during incubation and the relatively greater stability of tetracycline in these tests are revealed in the other 3 panels in Fig. 2. The chlortetracycline was the least stable and deterioration proceeded rapidly after 18 hours, so that only minor inhibition of the test organism was demonstrated in the plasmas after 24 hours and none at all was discernible at the 40-hour reading. The least changes occurred in the plasmas from the subjects who received tetracycline and only slightly greater changes were noted in the successive readings of the tests in the plasmas of those who had taken oxytetracycline.

The results of the assays of urine obtained during the first 24 hours after the third dose are shown in Table I; these results are based on readings made after incubation for 18 hours. All of the antibiotics appeared rapidly in the urine, but the greatest variations in the concentrations and amounts excreted were noted in the first 2 hours. The maximum concentrations were obtained in most instances in the 2-6 hour specimens, but high concentrations were still found in the next 6 hours, and in some subjects even during the second 12 hours. The total amount recovered in the urine during the first 12 hours was lowest in the 2 subjects who took the oxytetracycline tablets, and varied in the others between 9% and 21% of the administered dose. During the second 12 hours, the amount excreted also varied in different subjects between 1.3 and 6.5% of the dose, but one of the subjects who took the oxytetracycline tablets excreted 24.3% of the dose during this period. The specimens of urine obtained just before the dose are not listed in the table, but they showed activity equivalent to 0.06-0.25 µg/ml in almost all of the subjects; this was the result of the continued excretion of antibiotic from the preceding dose, which was given 5 days previously. These amounts, however, when calculated for the daily output, could account for excretion of only a fraction of 1% of the administered dose. Individual specimens of urine obtained after the first 24 hours from one of the subjects who took tetracycline showed a concentration of 0.5 µg/ml on the 9th day, but none detectable on the 10th day; in a second subject, the equivalent of 0.5 µg/ml of tetracycline activity was found on the 5th day and none was detected on the 9th day. Oxytetracycline was found in urine obtained on the 5th day in a concentration of 0.25 µg/ml in one, and 2.0 µg/ml in another, and none was detected in specimens from either of these individuals on the 9th day.

TABLE I. Urinary Excretion of 3 Tetracyclines in Normal Adults after Ingestion of 1.0 g.

Subject	Antibiotic*	Conc., μg/ml of urine, Interval after dose (hr) 0–2	2–6	6–12	12–24	mg of antibiotic excreted, Interval after dose (hr) 0–2	2–6	6–12	12–24	% of dose excreted 12 hr	24 hr
C	T–c	40	320	160	—	2.4	53.8	29.2	—	8.5	—
D	"	40	320	320	40	3.2	64.4	76.8	12.6	14.4	15.7
J	T–t	320	320	320	160	17.6	10.6	67.2	64.8	9.5	16.0
K	"	320	640	320	160	35.2	96.0	81.6	63.2	21.3	27.6
E	O–c	80	320	160	80	4.8	49.6	41.9	17.2	9.6	11.4
F	"	320	320	160	—	10.2	41.6	37.6	—	8.9	—
L	O–t	20	80	320	1280	1.1	9.2	49.6	243.2	6.0	30.3
M	"	20	80	80	80	1.1	11.6	15.6	32.8	2.8	6.1
A	C–c	160	320	40	—	22.4	55.0	20.0	—	9.7	—
B	"	10	160	640	—	0.9	40.0	67.2	—	10.8	—
G	C–t	160	320	160	80	16.0	52.8	62.4	42.0	13.1	17.3
H	"	160	320	320	320	36.8	89.6	48.0	83.2	18.4	25.8

* T = tetracycline; O = oxytetracycline; C = chlortetracycline; c = capsules; t = tablets. All were hydrochlorides except oxytetracycline tablet which was the free base.

In the second experiment, done several weeks later, only capsules of the hydrochlorides were given. Only 11 subjects were available, 3 receiving chlortetracycline and the other 2 analogs each being given to 4 subjects. Five of these subjects had participated in the earlier experiment, but none had taken any antibiotics in the preceding weeks. Blood was obtained before the dose and at 1, 2, 4, 8, 12, 16, and 24 hours. A control specimen of urine was obtained before the dose and complete collections were made throughout the first 48 hours; single specimens were then obtained daily until no antibiotic could be detected by the test read at 18 hours (*i.e.*, until there was less than 0.06 μg/ml). The results based on tests read after incubation for 18 hours are shown in Fig. 3 and Table II. In this experiment, the levels obtained with tetracycline and oxytetracycline appeared to be similar, although higher levels were more frequent and more sustained with the former. Plasma levels were slowest to rise and earliest and most rapid to fall in the subjects who took the chlortetracycline; the earlier and more rapid deterioration of this antibiotic in these tests as compared with the observations made in the first experiment, may have contributed to this result. Significant concentrations of antibiotic were sustained in the blood even as long as 24 hours after the dose of either tetracycline or oxytetracycline.

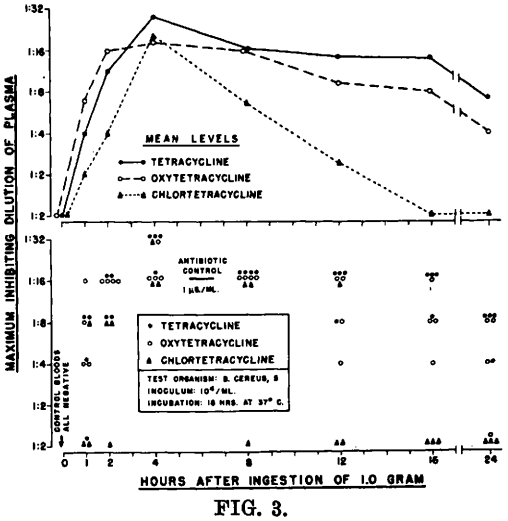

FIG. 3.

The urinary excretion of the 3 analogs during the first 24 hours, as shown in Table II, was similar to that observed in the first experiment. Similar variations were noted among subjects who received each of the 3 antibiotics, both with respect to the concentrations and the amounts excreted at different intervals. In this experiment, no inhibitory activity on the test organism was detected in the urine of any of the subjects just before taking the dose. The concentrations of antibiotic and the amounts excreted appeared to be lower after the first 24 hours in the subjects who had taken chlortetracycline. Excretion of the antibiotics continued in almost all the subjects through the 8th day; in 2 who took tetracycline, and in one who took oxytetracycline, some activity was still detected in urine obtained on the 10th day.

TABLE II. Urinary Excretion of 3 Tetracyclines after 1.0 g Orally Given as Capsules of the Hydrochlorides.

Subject	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Antibiotic	Tetracycline				Oxytetracycline				Chlortetracycline		
	Conc. in urine, μg/ml										
Before dose	0*	0	0	0	0	0	0	0	0	0	0
0- 2 hr	80	80	160	20	320	320	320	320	10	320	80
2- 4	320	640	160	160	320	160	320	640	80	320	320
4- 8	320	320	320	160	320	80	320	640	160	160	640
8-12	320	320	160	160	160	80	640	320	40	160	160
12-16	160	160	80	80	80	40	320	320	20	40	160
16-24	80	160	80	80	80	40	160	160	40	80	80
24-36	40	40	40	20	40	20	80	80	5	10	20
36-48	20	20	20	10	20	10	40	20	1	5	10
3 day	8.0	4.0	—	—	4.0	2.0	4.0	8.0	.5	.5	2.0
4	4.0	4.0	2.0	2.0	2.0	2.0	2.0	1.0	.06	.06	2.0
5	1.0	2.0	1.0	1.0	1.0	1.0	1.0	.5	.06	.13	.25
6	.5	.5	.13	.13	.25	.5	—	.25	0	.13	.13
7	.25	.25	.25	.25	.25	.25	.25	.13	0	0	.13
8	.13	.13	.13	.13	.13	0	.13	.13	0	.13	.06
9	.13	0	0	.13	.06	0	.13	.06	0	0	.06
10	.06			.13	0		.06	0			
11	0			.06	0		0	0			
	% of dose excreted										
12 hr	12.4	17.8	14.1	7.9	10.1	5.8	17.9	16.5	4.6	14.1	19.4
24	18.5	23.0	18.5	10.2	13.8	8.0	26.6	24.5	6.8	17.6	26.7
48	21.0	25.2	22.2	11.5	17.1	9.5	31.3	26.8	7.3	18.7	28.0

* <0.06 μg/ml.

TABLE III. Toxic Effects Observed in 12 Normal Adult Male Subjects after Ingestion of 1.0 g of Each of 3 Tetracyclines.

Subject	Antibiotic: Tetracycline	Oxytetracycline	Chlortetracycline
	Capsules		
A	*1. None	2. None	3. None
B	1. "	2. Epigastric distress, began in 24 hr, lasted 3 days	3. Epigastric distress & nausea after 24 hr, lasted 48 hr
C	3. "	1. Diarrhea, 4 days	2. None
D	3. "	1. None	2. Bulky stools
E	2. "	3. "	1. None
F	2. 3-4 bulky stools	3. Flatulence	1. "
	Tablets		
G	1. None	2. Diarrhea	3. None
H	1. "	2. Diarrhea, tenesmus, lasted 3 days	3. Tenesmus, diarrhea next day
J	3. "	1. Nausea & diarrhea, 1 day	2. None
K	3. "	1. Flatulence for 36 hr, then diarrhea	2. Diarrhea, ? from previous dose, lasted 36 hr
L	2. "	3. None	1. Nausea, mild abdominal distress for 48 hr
M	2. "	3. "	1. Bulky stools, 24 hr

* Number represents the order of administration, 4 to 7 days apart.

Untoward effects. The symptoms experienced by the subjects and attributable to the antibiotics during the first experiment are listed in Table III. These findings confirm those observed in clinical studies which indicated that the major untoward effects of each of the tetracyclines are referable to the gastrointestinal tract and that, of these, diarrhea is the most serious; they also confirm the observations made in this hospital that such gastrointestinal effects are most frequent with oxytetracycline and least frequent with tetra-

cycline(2). In the present experiment there were essentially no untoward effects from tetracycline, only one of the subjects having passed 3 or 4 bulky stools on the day after the dose. Diarrhea, flatulence or epigastric distress in various combinations were experienced by 7 of the subjects who took oxytetracycline and by 4 of those who took chlortetracycline, and 2 additional subjects passed some bulky stools after doses of the latter. In 3 of the subjects who manifested gastrointestinal symptoms from chlortetracycline including 2 with diarrhea, the chlortetracycline was given after oxytetracycline and similar symptoms were experienced with the previous dose.

In the second experiment one of the subjects who took oxytetracycline had 4 watery bowel movements on that day and 2 to 4 similar watery movements during each of the next 4 days; this individual had not participated in the earlier experiment. One subject experienced mild nausea immediately on taking the tetracycline capsules and another after taking the chlortetracycline, both on an empty stomach; in both subjects the nausea cleared promptly after they began to eat breakfast and no other symptoms were experienced by these or by any of the other subjects.

Discussion. In the previous paper(1) it was shown that the 3 tetracyclines have very similar activity against common pathogenic bacteria, but that they differed in their stability and, to some extent, also in their activity against individual strains or against certain species of bacteria. The relative instability of chlortetracycline under the conditions of the *in vitro* inhibition tests, or in assays of the antibiotic in body fluids, may be of paramount importance in making comparisons of the absorption and excretion of the 3 tetracyclines. In the data presented here, this is clearly brought out in comparing the blood levels. That the same phenomenon is not necessarily true, at least to the same extent, *in vivo,* is apparent from the fact that chlortetracycline continues to be excreted for several days after a single dose, and in concentrations not markedly dissimilar from those observed with the other 2 analogs which are more stable *in vitro.* It has also been shown that in embryonated eggs, chlortetracycline does not deteriorate nearly so rapidly as it does in culture media kept at the same temperature(3), and a substance has been found in egg yolk which apparently protects the chlortetracycline from deterioration(4). It seems not unlikely, therefore, that similar protection against deterioration is available in tissues and body fluids in which, despite their slightly alkaline reaction, chlortetracycline appears to persist in active form for several days. On the whole, however, blood levels from oral tetracycline appear to be somewhat higher and more sustained and its excretion appears to be slightly prolonged over that observed following similar oral doses of oxytetracycline and chlortetracycline.

Conclusions. Blood levels after ingestion of a single dose of 1 g of tetracycline are somewhat higher and better sustained than those resulting from a similar dose of either oxytetracycline (Terramycin) or chlortetracycline (Aureomycin). High concentrations appear rapidly in the urine after ingestion of each of these 3 analogs and persist over a period of 48 hours. After that, appreciable concentrations of these antibiotics continue to be excreted for more than a week after a single oral dose of 1.0 g. Untoward gastrointestinal symptoms, particularly diarrhea, were frequent after a single oral dose of 1.0 g of oxytetracycline, they were observed, but less frequently after chlortetracycline, and did not occur after the same dose of tetracycline.

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