synthesis due to metabolic imbalances brought about by the rapid growth conditions employed in this study.

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α-6-Deoxyoxytetracycline III. Total and Unbound Antibiotic Serum Concentrations After Oral Administration to Mice. (32485)

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The new tetracycline, α-6-deoxyoxytetracycline (DOOTC)* has demonstrated a marked chemotherapeutic advantage over other tetracyclines when administered orally to experimentally infected mice. For example, DOOTC was significantly more active than other tetracyclines against infections produced by the intraperitoneal inoculation of *Staphylococcus aureus* 5 mp, *Pasteurella multocida*, and *Streptococcus pyogenes* C 203 (1). In addition, DOOTC afforded 2 to 4 times greater protective activity than other tetracyclines after oral administration to mice in (a) pre-infection and (b) post-infection protocols(2). The studies reported in this paper suggest that the superior performance of DOOTC in such experiments depends upon the achievement of higher free-drug levels in serum and other body fluids.

Materials and methods. The antibiotics, *i.e.*, α-6-deoxyoxytetracycline, 6-methylene oxytetracycline (MOTC)‡, 6-demethylchlortetracycline (DMCT)§, and tetracycline (TC)¶ were used in these studies as the hydrochlorides and were of research quality. All tetracyclines were administered orally to mice in a diluent consisting of water and 1% carboxymethylcellulose.

At various times after antibiotic administration to a large number of 20-25 g Swiss Albino mice (Blue Spruce Farms, Altamont, N. Y.), a group (7 mice) was removed and sacrificed by either decapitation or exsanguination from the subclavian artery. Serum was separated from the pooled blood sample at refrigerator temperature. If not biologically assayed on the day collected the serum sample was kept frozen until used.

Bioassays of sera were performed using the common paper disc or cup-plate technique with *Bacillus cereus* var. *mycoides* as test organism(3). Standard curves were prepared using either pooled normal mouse serum or a solution containing sufficient bovine albumin to compensate for serum binding. Serum samples were used either undiluted, diluted with normal mouse serum, or diluted with the albumin solution.

The serum concentration studies in the mouse were replicated, dependent upon the antibiotic studied, from 6 to 12 times. Therefore, the values in the Tables usually represent the mean calculated from 6 to 12 different pooled sera samples. The standard error of the mean has also been calculated. These data

* Generic name, doxycyline. The trade mark of Chas. Pfizer and Co., Inc. is Vibramycin.

‡ Registered trade mark of Chas. Pfizer and Co., Inc., is Rondomycin.

§ Registered trade mark of Lederle Laboratories, American Cyanamid Co., Inc., is Declomycin. I wish to thank Dr. B. W. Corey, Medical Director of Lederle Laboratories, Pearl River, N. Y. for the supply of DMCT.

¶ Registered trade mark of Chas. Pfizer and Co., Inc. is Tetracyn.

in Table II are presented as total antibiotic content in the sera.

The per cent binding of DOOTC and the other tetracyclines to mouse serum proteins was determined by the microbiological plate procedure described by Scholtan and Schmid for penicillins(4) and tetracyclines(5). Briefly, a comparison was made between zone sizes produced against *B. cereus* var. *mycoides* by equal concentrations of each antibiotic in, respectively, pooled normal mouse serum (pH 7.4) and pH 7.4-buffered-saline. The inhibition zone produced by a tetracycline in buffered saline is larger than that produced when in mouse serum and such difference in inhibition area is interpreted as a consequence of serum binding, *e.g.*, (a) the tetracycline in buffer, which is present as unbound antibiotic, has a faster diffusion rate than the serum-tetracycline combination when tested in agar and/or only that portion of tetracycline in the serum which is present as unbound antibiotic can exert antimicrobial activity. Standard curves are drawn on semilogarithmic graph paper after plotting points representing zone sizes and corresponding known antibiotic concentrations. Both lines run at a certain distance from each other and are virtually parallel. A common zone size was selected from the standard curves as determined in buffered saline and in mouse serum, and the antibiotic concentrations yielding this identical zone were substituted in the following formula:

$$\% \text{ bound antibiotic in mouse serum} = \frac{\text{Antibiotic concentration in serum} - \text{that in buffered saline}}{\text{concentration in serum}} \times 100.$$

The per cent serum binding in any single experiment was obtained by calculating the average values obtained for 3 different common zone sizes. The per cent values presented in Table I are means of 5 to 10 different experiments. The standard error of the mean is also presented.

For the determination of urinary recovery of these antibiotics, groups of 15 mice housed in plastic metabolism cages were administered either intravenous or oral dosages of 10 mg/kg. At various times during the study, usually at 6, 24, 28, 48, and 72 hours, feces-free urine samples were collected, volume recorded, and were biologically assayed for the appropriate tetracycline content. In each instance, standard curves and any necessary sample dilutions were prepared in normal mouse urine. The per cent of tetracycline recovered of drug administered by each route was calculated. In turn, the per cent recovery after intravenous administration was divided by the per cent recovery after oral administration to give an estimate of efficiency of oral absorption. These data were replicated in 3 to 6 different experiments. Mean values and standard error of the means were calculated and are presented.

TABLE I. Per Cent Binding of DOOTC and Other Tetracyclines to Mouse Serum Proteins.*

Tetracycline	% Binding to mouse serum proteins, mean ± standard error
DOOTC	69.6 ± 2.62
MOTC	69.7 ± 2.08
TC	54.8 ± 2.21
DMCT	73.3 ± 1.88

* Determined by microbiological plate technic of Scholtan and Schmid(4,5). Treatment time of 2 hr at 37°C before cups in plate filled with antibiotic-mouse serum or antibiotic-buffered saline mixture. Zones read after 20 hr at 37°C.

Results and discussion. The values for average per cent binding of the tetracyclines in mouse serum are presented in Table I. Values for TC and DMCT are similar to those reported for human serum, however, the DOOTC and MOTC figures in mouse serum are somewhat lower (10-15%) than those reported for human serum(6). The mean binding values presented in Table I and mean serum concentrations in Table II were used to calculate the unbound antibiotic concentrations in serum, *i.e.*, total antibiotic—portion bound = unbound portion, as presented in Table III.

Tables II and III present means of serum concentrations as, respectively, total and unbound antibiotic for DOOTC and the other tetracyclines tested after a single oral dose of 12.5 mg/kg to mice. Mean values for DOOTC at a 6.25 mg/kg dose also are shown for comparative purposes. In terms of both total and unbound antibiotic mean concentrations, two points are evident: (a) DOOTC produced

TABLE II. Serum Concentrations as Total Antibiotic of DOOTC, MOTC, DMCT, and TC in the Mouse after Oral Antibiotic Administration.

Sample hours	Serum concentrations, mean ± standard error of total antibiotic, mcg/ml, after oral dosage (mg/Kg)				
	DOOTC		MOTC	DMCT	TC
	12.5	6.25	12.5	12.5	12.5
0.5	2.99 ± .25	1.23 ± .25	.72 ± .11	.54 ± .09	.83 ± .14
1	2.99 ± .39	1.34 ± .45	.79 ± .16	.84 ± .09	.98 ± .06
2	2.32 ± .34	1.04 ± .28	.60 ± .20	.41 ± .10	.75 ± .08
3	1.98 ± .26	1.02 ± .23	.66 ± .14	.25 ± .10	.49 ± .10
4	1.44 ± .20	.64 ± .26	.46 ± .08	.15 ± .05	0
6	1.15 ± .17	.44 ± .15	.31 ± .06	0	
8	.92 ± .23	.26 ± .08	.19 ± .08		
9	.30 ± 0	.16 ± 0	0		
10	.13 ± 0	0			
11	.16 ± 0				
12	0 0				

TABLE III. Serum Concentrations as Unbound Antibiotic of DOOTC, MOTC, DMCT, and TC in the Mouse After Oral Administration.*

Sample hr	Serum concentrations as unbound antibiotic, mcg/ml, at hr calculated from Table 1 and 2				
	DOOTC		MOTC	DMCT	TC
	2.5	6.25	12.5	12.5	12.5
0.5	.90	.37	.22	.15	.37
1	.90	.40	.24	.23	.44
2	.70	.31	.18	.11	.34
3	.57	.31	.20	.07	.22
4	.44	.19	.14	.04	
6	.35	.13	.09		
8	.28	.08	.06		
9	.09	.05			
10	.04				
11	.05				
12	0				

* Calculated from mean value for serum protein binding in Table 1 and mean value for total antibiotic found in serum after oral administration as in Table 2.

markedly higher and (b) longer lasting serum activity than did the other tetracyclines. For example, in the samples showing highest antibiotic serum levels (1 hour post injection), the mean concentration, as total antibiotic, of DOOTC was 3 to 4 times higher than those of the other tetracyclines. In terms of unbound antibiotic (Table III), mean values of DOOTC at peaking were again about 2 to 4 times higher than those of the other tetracyclines. As DOOTC activity is obviously retained in the serum for a longer time than are the other tetracyclines, such comparison becomes more pronounced in the later time samples. Measurable DOOTC, as total and unbound antibiotic, was still detectable 11 hours after a single oral dose of 12.5 mg/kg. MOTC activity was detectable through 8 hours; DMCT, for 4 but not 6 hours; and TC, for 3 but not 4 hours.

Even when administered at one-half the oral dosage of the other tetracyclines, DOOTC produced higher means of total and, in general, higher means of unbound antibiotic concentrations than did MOTC, TC, and DMCT (Tables I and II). The calculated unbound concentrations as means of TC (at 12.5 mg/kg) in the early time samples were somewhat higher than unbound DOOTC mean concentrations after dosage of 6.25 mg/kg. However, in duration of serum activity, DOOTC at 6.25 mg/kg was superior to TC and DMCT and somewhat better than MOTC.

Although it is common in the evaluation of new antimicrobial agents to present serum levels in terms of total antibiotic, the con-

centration existing as unbound drug is emphasized here. Admittedly protein binding does have an important effect upon drug pharmacodynamics including distribution into body tissues and fluids, urinary excretion, serum retention and possible drug metabolism. However, the exact role and contribution of each of the above to chemotherapeutic efficacy are not clearly understood. In contrast, it is generally believed that only the unbound portion of a drug exerts direct chemotherapeutic activity. This has been shown for medicinal dyes, alkaloids, and organic acids as well as for penicillins, sulfonamides, and other antimicrobial agents (7,8,9,10). Therefore, it is clear that among the primary requisites for the demonstration of *in vivo* protection must be a concentration of unbound antibiotic high enough and in contact long enough with the pathogen to produce effective inhibition. Using the *in vitro* MIC (minimum inhibitory concentration, μg/ml) as a readily-available criterion of inherent antibiotic susceptibility of an organism, one can predict *in vivo* activity if the serum concentration of unbound antibiotic reaches or exceeds that value. Previous studies have indicated the MIC's of DOOTC, MOTC, TC, and DMCT for the 3 organisms used in successful experimental infection studies to range from 0.1 to 0.2 for *S. aureus*, 0.03 to 0.04 for *S. pyogenes* C 203, and 0.16 to 0.30 for *P. multocida*(1). As shown in Table III, the unbound serum concentrations of DOOTC and the other tetracyclines exceed or are equal to these MIC values after an oral dose of 12.5 mg/kg. Furthermore, DOOTC produced serum levels of unbound antibiotic greater in concentration and of longer duration than the other tetracyclines even after administration at only one-half their dosage. The higher (at least 2-fold or greater) chemotherapeutic efficacy on a mg/kg basis of DOOTC over the other tetracyclines is explained readily on this basis.

As discussed recently by Kunin, serum concentrations at any one time are a consequence of a variety of pharmacodynamic properties including absorption from the gastrointestinal tract(11,12). Therefore, a comparison of serum concentrations of drug achieved after identical oral dosage will tend to ignore differences in drug distribution, excretion, and possible metabolism, and is not necessarily indicative of the superior gastrointestinal absorption of one of them. A method that better distinguishes between absorption efficiency and the other pharmacodynamic properties mentioned above is to determine the ratio between urinary recoveries of drug after oral and intravenous administration at the same dosage. Inherent in the method is the assumption that once absorbed, the drug will be handled by the animal in the same manner (11,12). Thus, such studies, which will be reported in detail later, were initiated with DOOTC and the other tetracyclines to assess the role of gastrointestinal absorption in the production of serum concentrations of these antibiotics. The summary of such data (Table IV) offers convincing evidence that DOOTC is

TABLE IV. Efficiency of Oral Absorption of DOOTC and other Tetracyclines in the Mouse.*

Tetracycline	% Oral absorption mean ± standard error
DOOTC	74 ± 5.4
MOTC	22 ± 8.0
TC	16 ± 1.2
DMCT	19 ± 7.9

$$\frac{\text{* Urine Recovery as \% of Oral Administration Dose}}{\text{Urine Recovery as \% of iv Administered Dose}} = \text{\% Oral Absorption}$$

Urines collected over a 72-hr period.

more efficiently absorbed from the gastrointestinal tract of mice than are the other tetracyclines, *i.e.*, the 74% value for DOOTC is approximately 3.5 to 4.5 times greater than any obtained with the other tetracyclines.

Summary. 1. The results of mouse serum level studies are presented in terms of total and unbound antibiotic concentrations. DOOTC administered at one-half the oral dosage of MOTC, TC, and DMCT produced higher and longer lasting total and unbound drug concentrations than the latter antibiotics. 2. It is suggested that the previously reported chemotherapeutic superiority of DOOTC over these drugs results from the achievement and maintenance of superior unbound drug levels. 3. The more efficient oral absorption of DOOTC over the other tetracyclines (3.5-4.5

times) in the mouse was demonstrated by comparing cummulative urinary excretion ratios obtained after oral and intravenous drug administration.

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DNA Biosynthesis in Monkey Kidney Cells Infected With PARA (SV40)-Adenoviruses.* (32486)

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Recent studies have shown that a human adenovirus type 7 which grows in African green monkey kidney (GMK) cell cultures carries a portion of the SV40 genome(1-3). The virus population was found to consist of at least two types of particles(4,5), one being a normal adenovirus and the other containing the defective SV40 genome in an adenovirus capsid; the replication of these particles is interdependent in simian cells(6). This SV40-adenovirus 7 "hybrid" was called PARA-adenovirus 7(7).

Physical separation of the PARA particle (containing the SV40 genome) and the respective adenovirus in a mixed population has been unsuccessful(6,8-10). The SV40 determinants carried by the PARA-adenovirus include those coding for the induction of SV40 tumor (T) antigen(2,3) and transplantation rejection antigens(11). These properties can be transferred to other adenovirus types(7,12) and such transcapsidants breed true. Although PARA and adenovirus virions replicate in parallel, information on biosynthesis of deoxyribonucleic acid (DNA) by the infected cells has not yet been obtained. The present investigation was therefore undertaken to follow the synthesis of DNA in cultures of GMK cells inoculated with PARA-adenovirus 7, or with one of the transcapsidants PARA-adenovirus 2 or PARA-adenovirus 12, and to characterize the DNA from the infected cultures.

Materials and methods. Cells. GMK cells were grown in 16-oz bottles using Melnick-Hanks' lactalbumin hydrolysate medium with 2% calf serum as previously described(13). In some instances, GMK cells were also grown in 60 mm plastic petri dishes or on 22 mm square coverslips placed in the petri dishes. After 10-12 days of growth, the primary cultures were nourished with Eagle's minimal medium for 24 hours before the experiments were started.

Viruses. Stocks of PARA-adenovirus 7 and its transcapsidants were prepared in GMK cells as described(7). In each experiment, the virus was allowed to adsorb for 1.5 hours at 37C. The input multiplicity for PARA was adjusted to 1 to 3 plaque-forming units

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