Factors Affecting Serum Oxytetracycline Levels in Beef Calves

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Abstract □ Fifteen Aberdeen Angus steers, 295–364 kg, were dosed with either 4.4 or 11 mg of oxytetracycline hydrochloride/kg im. The antimicrobial activity of the serum was determined periodically, and the resulting data were treated statistically to determine the sources of variation. Variance in serum levels of oxytetracycline activity was attributed to dose, time of bleeding, order of dosing, animal, and assay. The total variance component was proportionately greater for the 11-mg/kg dose than for the 4.4-mg/kg dose. Animal variance increased with the higher dose level of oxytetracycline. The influence of dose on serum level was tested by applying a t test to the mean serum levels and their standard deviations at each bleeding time. The 4.4- and 11-mg/kg serum levels were significantly different ($p < 0.01$) at all bleeding times. The 4.4-mg/kg serum levels multiplied by 2.5 were not significantly different ($p < 0.05$) from the 11-mg/kg serum levels at all bleeding times.

Keyphrases □ Oxytetracycline—factors affecting serum levels, calves □ Drug levels, serum—oxytetracycline, factors causing variance, calves □ Antibacterials—oxytetracycline, factors affecting serum levels, calves

Blood or serum drug levels are often utilized to determine the absorption, availability, or excretion rates of certain drugs and chemical compounds. These levels have also been used to assess the bioavailability or bioequivalency of similar drug products. Previous reports (1–5) found undesirable variability in the drug level data from both human and animal studies. To compensate for these variables, large numbers of animals or humans may be required.

Certain parameters, such as route of administration and drug formulation, obviously can influence variability. Other factors, such as animal, dose period, dose, and assay variance, have been less well defined as sources of variation. More insidious factors, such as ion chelation and protein binding, also may contribute to the animal variance factor and, therefore, to the overall variance component. The physical preparation of analytical samples may introduce additional variations in results.

Since it is not feasible to control all potential variables in a given blood level study, it was deemed advisable to determine, directly or indirectly, the extent that these factors contribute to the overall variance component in a study of serum levels of an antibiotic (oxytetracycline) in animals (calves). Five critical factors, comprising a major portion of the variance component, were studied. These factors were animal, dose, order of dosing, time of bleeding, and assay.

EXPERIMENTAL

Dosing and Animal Handling—Fifteen purebred Aberdeen Angus steers, 295–364 kg, were divided into two groups of seven and eight calves each. Each group was further subdivided into two treatment levels. One group was given 4.4 mg, and the other was given 11 mg of oxytetracycline hydrochloride[1]/kg im. Treatments were administered one time to each animal for each sampling period. The dose was adjusted according to individual weights at each treatment interval.

One group of animals was dosed and bled on Day 1, and the other group was dosed and bled 1 week later. This sequence of events was defined as Period 1. Seven weeks later, the groups were reversed according to a double-crossover design, and the study was repeated. These events were defined as Period 2.

The animals were maintained in a drylot paddock area with free access to feed and water. They were observed daily for any deviation from normal health. No clinical illness was observed during the trials.

Blood Sampling—Blood samples were collected from the jugular vein immediately prior to treatment and at intervals of 0.5, 1, 2, 4, 6, 8, 12, 15, and 18 hr after Period 1 dosing. After Period 2 dosing, samples were taken at 2, 4, 6, 8, and 15 hr. Blood samples were protected from direct sunlight and refrigerated immediately after collection. Serum was harvested from the samples as rapidly as clot retraction occurred or generally within the hour. The serum samples were then frozen and protected from light until assayed, which generally occurred within 48 hr to 1 week. Quadruplicate assays were performed on each serum sample.

Assay—Concentrations of oxytetracycline in serum were determined by using the microbiological cylinder plate assay method (6) with *Bacillus cereus* var. *mycoides* (ATCC 11778) as the test organism.

Statistical Analysis—The data were treated statistically using a computer[2] with a statistical analysis system (7). The following adjustments and assumptions were made:

1. Only bleeding times common to both periods of the experiment (*i.e.*, 2, 4, 6, 8, and 15 hr following drug administration) were treated.
2. From pharmacokinetic considerations, it was assumed that time of bleeding would influence the serum levels following drug administration in a definable way (8); this parameter was not included in the statistical analyses. It was anticipated that the influence of time could be depicted as plots of the mean serum levels ± *SD versus* time. These plots for the 4.4- and 11-mg/kg doses are shown in Fig. 1.
3. It was assumed, *a priori*, that dose would influence the serum levels; this parameter was not included in the analysis of variance. It was anticipated that the influence of dose could be examined by applying the Student t test to the mean serum levels for the two doses at each time point.
4. It was assumed that the order of dosing (*i.e.*, 4.4 followed by 11 mg/kg or vice versa) might influence the results but that the division of the animals into four groups and the handling of the groups on different days would not have an influence.
5. It was assumed that the serum assays were carried out in a perfectly random manner.

The data were divided into units consisting of the assay results for all animals at a single bleeding time following a single dose, and the variance components due to order of dosing, animal, and assay were determined. The "animal" parameter was "nested" within the "order of dosing" parameter. The variance component of each parameter and the total variance component are defined by the following equations (9):

$$\text{variance component}_{\text{assay}} = \sigma^2 = \text{mean square}_{\text{assay}} \quad \text{(Eq. 1)}$$

$$\text{variance component}_{\text{animal}} = \sigma^2 \text{ animal} = \frac{\text{mean square}_{\text{animal}} - \text{mean square}_{\text{assay}}}{\text{number of replicates per animal}} \quad \text{(Eq. 2)}$$

$$\text{variance component}_{\text{order}} = \sigma^2 \text{ order} = \frac{\text{mean square}_{\text{order}} - \text{mean square}_{\text{animal}}}{\text{number of replicates per order}} \quad \text{(Eq. 3)}$$

$$\text{total variance component} = \sigma^2 + \sigma^2 \text{ animal} + \sigma^2 \text{ order} \quad \text{(Eq. 4)}$$

[1] Liquamycin injectable (50 mg/ml), Pfizer Inc., New York, N.Y.

[2] IBM 360/50.

Table I—Effect of Assay, Animal, and Order of Dosing on Mean Serum Oxytetracycline Levels in Calves [a]

Bleeding Time, hr	Mean Serum Level, μg/ml	Total Variance Component	Variance Component (V.C.) and Percent of Total Variance Component Due to: Assay V.C.	Assay %	Animal V.C.	Animal %	Order of Dosing V.C.	Order of Dosing %
			Dose = 4.4 mg/kg im					
2	1.85	0.36	0.05	14.7	0.31	84.2	0.004	1.2
4	2.33	0.51	0.19	38.2	0.24	47.1	0.07	14.6
6	2.68	1.76	1.00	56.8	0.38	21.8	0.38	21.4
8	2.49	0.97	0.43	44.6	0.43	44.5	0.11	10.9
15	2.03	1.21	0.80	65.8	0.00	0.0	0.42	34.2
Average		0.96	0.49		0.27		0.19	
Average, %		100.0	51.5		28.2		20.3	
			Dose = 11 mg/kg im					
2	4.15	2.67	0.22	8.4	1.21	45.4	1.23	46.2
4	5.62	3.16	1.38	43.8	0.52	16.5	1.26	39.7
6	6.23	9.41	3.80	40.4	2.74	29.1	2.87	30.5
8	5.47	2.92	1.08	37.0	1.38	47.2	0.46	15.9
15	4.78	6.73	2.69	40.0	1.19	17.7	2.85	42.3
Average		4.98	1.84		1.41		1.73	
Average, %		100.0	36.9		28.3		34.8	

[a] All fractions were calculated using six decimal places and rounded to two or three figures.

RESULTS AND DISCUSSION

The results are given in Table I in terms of mean serum levels for individual bleeding times following a given dose with variance components due to assay, animal, and order of dosing. Only results common to both periods (*i.e.*, 0, 2, 4, 6, 8, and 15 hr) are included. The influences of dose and time of bleeding were not included in the statistical analyses.

The results showed that the absolute value of the total variance component was proportionately greater for the 11-mg/kg dose than for the 4.4-mg/kg dose. The percent contribution of the animal component was the same in each case, indicating that the animal groups were well matched. As expected, the fraction of the variance component attributable to the assay was greater for the 4.4-mg/kg dose, since this dose resulted in lower serum levels, which presumably would produce more variable assays. The percent contribution of the order of dosing was greater after the higher dose.

Unfortunately, the bleeding schedule was insufficient to define the entire serum level–time curves. If this had been possible, the influence of dose on the serum levels could have been expressed in terms of the areas under the curves, which would have revealed any differences in the absorption efficiencies of the two doses. If both doses were equally well absorbed, the area under the 11-mg/kg curve would be exactly 2.5 times the area under the 4.4-mg/kg curve (10). Definition of the entire serum level–time curves would also have revealed any differences in absorption or elimination kinetics of the two doses.

In the absence of complete serum level–time curves, the influence of

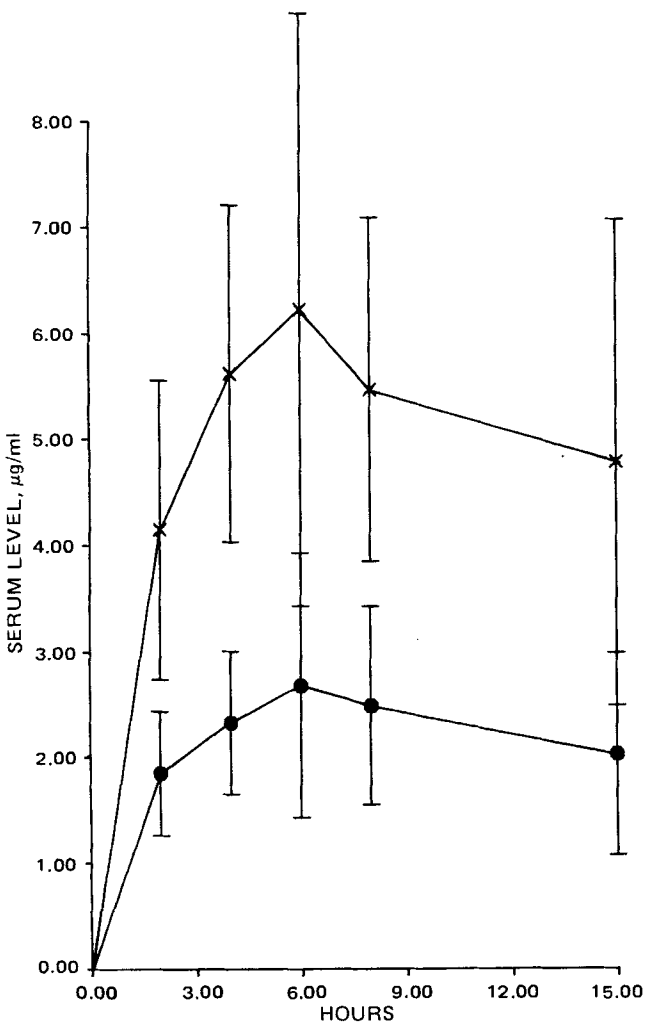

Figure 1—*Mean serum levels (micrograms per milliliter ± SD) of oxytetracycline in 15 Aberdeen Angus calves following intramuscular administration of 4.4- (●) and 11-(×) mg/kg doses. Each point contains results from quadruplicate assays from 15 serum samples, except that 14 samples were run for the 15-hr point of the 4.4-mg/kg dose.*

Table II—*t* Test of Difference in Mean Serum Oxytetracycline Levels in Calves (4.4 *versus* 11 mg/kg)

Bleeding Time, hr	4.4 mg/kg Mean Serum Level, μg/ml	4.4 mg/kg *SD*	11 mg/kg Mean Serum Level, μg/ml	11 mg/kg *SD*	t [a]
2	1.85	0.59	4.15	1.41	11.66
4	2.33	0.68	5.62	1.59	14.74
6	2.68	1.25	6.23	2.80	8.97
8	2.49	0.94	5.47	1.62	12.32
15	2.03	0.96	4.78	2.29	8.58

[a] All serum levels were significantly different at $p < 0.01$.

Table III—*t* Test of Difference in 2.5 × Mean Serum Oxytetracycline Levels in Calves (4.4 *versus* 11 mg/kg)

Bleeding Time, hr	2.5 × 4.4 mg/kg Mean Serum Level, μg/ml	$SD \times (2.5)^{1/2}$	11 mg/kg Mean Serum Level, μg/ml	11 mg/kg *SD*	t
2	4.63	0.93	4.15	1.41	2.20[a]
4	5.83	1.08	5.62	1.59	0.85[b]
6	6.70	1.98	6.23	2.80	1.06[b]
8	6.23	1.49	5.47	1.62	2.67[a]
15	5.08	1.52	4.78	2.29	0.86[b]

[a] Serum levels were not significantly different at $p < 0.05$. [b] Serum levels were not significantly different at $p < 0.01$.

dose on serum level can be defined by comparing the mean serum levels for the two doses at each bleeding time (10). A t test was applied to the mean serum levels and their standard deviations, and the results (Table II) showed that the mean serum levels were significantly different ($p < 0.01$) at each bleeding time.

A t test was also performed between the serum levels following the 11-mg/kg dose and 2.5 times the serum levels following the 4.4-mg/kg dose. There was no significant difference ($p < 0.05$) between the serum levels following the 11-mg/kg dose and 2.5 times the serum levels following the 4.4-mg/kg dose (Table III). These results suggest that serum levels are directly proportional to dose levels.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 12, 1975, from the *Division of Veterinary Medical Research, Bureau of Veterinary Medicine, Food and Drug Administration, Beltsville, MD 20705, †The National Center for Antibiotic Analysis, Bureau of Drugs, Food and Drug Administration, Washington, DC 20204, and the §College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Accepted for publication September 22, 1976.

The authors thank Dr. D. Kratzer of the Department of Statistics, University of Kentucky, for helpful discussions and suggestions concerning the statistical analysis system.

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Anomalous Chemical Shifts of Methyl Groups of 2,4-Dimethylbenzo[g]quinoline

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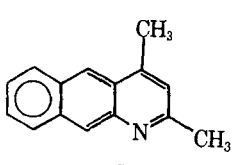
Abstract □ The chemical shifts of the methyl groups of 2,4-dimethylbenzo[g]quinoline are defined with respect to concentration, showing that the methyl resonances are reversed from their expected positions in concentrations normally used in NMR spectroscopy. The phenomenon is explained in terms of the probable "fixation" of bonds in the hetero ring.

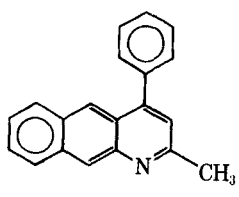
Keyphrases □ 2,4-Dimethylbenzo[g]quinoline—NMR spectrum, chemical shifts of methyl groups defined, effect of concentration □ NMR—spectrum, 2,4-dimethylbenzo[g]quinoline, chemical shifts of methyl groups defined, effect of concentration □ Quinoline, substituted—2,4-dimethylbenzo[g]quinoline, NMR spectrum, chemical shifts of methyl groups defined, effect of concentration

During a study of the possibility of converting 2,4-dimethylbenzo[g]quinoline (I) to benzo[g]cinchoninic acid, large concentration chemical shifts for the methyl groups of I were observed. At low concentrations (3.4×10^{-2} M), the absorptions for the methyl groups appeared as a doublet at δ 2.76 (J = 1.2 Hz) and as a singlet at δ 2.69. As the concentration of I was increased, the expected upfield shifts of the methyl groups occurred, but the downfield methyl resonance showed a larger upfield shift than the methyl resonance at δ 2.69. Therefore, at a concentration of 2.74×10^{-1} M, the two methyl signals had overlapped to produce a single resonance at δ 2.64. When the concentration was increased to 6.4×10^{-1} M, a singlet at δ 2.57 and a doublet at δ 2.5 (J = 1 Hz) were observed.

The classical assignment of the downfield resonance to the 2-methyl of I cannot be accommodated by this information. The chemical shift of the 2-methyl group would be expected to remain relatively constant as the concentration of I is increased while the 4-methyl group would be expected to have the greatest shift due to solute–solute interactions (1). This observation led to assignment of the resonance at δ 2.76 (J = 1.2 Hz) (3.4×10^{-2} M) to the 4-methyl group and at δ 2.69 to the 2-methyl group of I.



I



II

EXPERIMENTAL

Samples were weighed on a microbalance[1] and then diluted to volume with benzene-d_6 or spectrograde carbon tetrachloride containing 1% tetramethylsilane as an internal standard. The NMR spectra were obtained using a 60-MHz spectrometer[2] equipped with a double-resonance accessory.

2,4-Dimethylbenzo[g]quinoline (I) was prepared as reported previously

[1] Cahn RTL.
[2] Perkin-Elmer R12-A.