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THE DISTRIBUTION OF TETRACYCLINES IN TISSUES OF DOGS AFTER REPEATED ORAL ADMINISTRATION

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ABSTRACT

SCHACH VON WITTENAU, M. AND C. S. DELAHUNT: The distribution of tetracyclines in tissues of dogs after repeated oral administration. J. Pharmacol. **152:** 164–169, 1966. The concentrations of five tetracyclines—oxytetracycline, OTC (Terramycin); 6-methyleneoxytetracycline, MOTC (methacycline, Rondomycin); 6-demethylchlortetracycline, DMCT (Declomycin); α-6-deoxyoxytetracycline, DOOTC (doxycycline); and 6-demethyl-6-deoxytetracycline, DMDOTC—in canine tissues were determined after repeated oral administration of these drugs. It was found that the distribution of the antibiotics between tissue depots and interstitial fluid correlates well with the partition coefficients in chloroform and water, a measure of the lipid solubilities of the compounds. Although two tetracyclines may be similarly distributed in accordance with their like lipid solubilities, they will show vastly different total serum concentrations if they differ in serum protein binding. The correlation of drug serum concentrations achieved with tetracyclines in humans and dogs in discussed.

The excretion and distribution in body fluids of several tetracyclines after a single intravenous administration to dogs were determined in a previous study (Schach von Wittenau and Yeary, 1963). The results obtained were rationalized on the basis of serum protein binding and the lipid solubilities of the tetracyclines as determined by their partition between chloroform and water. It was inferred that a highly lipid-soluble tetracycline will be bound reversibly in tissue depots to a greater extent than a less lipid-soluble analog. Furthermore, as long as pKa values and metal-complexing powers are comparable, the ratio (drug bound to tissues)/(drug in interstitial fluid) would be dependent upon the lipid solubility of a tetracycline derivative. Although this ratio could be the same for two derivatives, the drug with the higher affinity for serum proteins would show higher concentrations in serum. Conversely, two similar compounds showing equal concentration in serum may differ in distribution between tissues and interstitial fluid.

This paper explores this concept further and reports the distribution in canine tissues of oxytetracycline, OTC (Terramycin); 6-methyleneoxytetracycline, MOTC (methacycline, Rondomycin); 6-demethylchlortetracycline, DMCT (Declomycin); α-6-deoxyoxytetracycline, DOOTC (doxycycline); and 6-demethyl-6-deoxytetracycline, DMDOTC, after repeated oral administration. Their structures are presented in figure 1.

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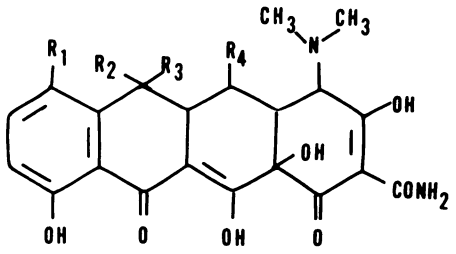
The compounds under discussion have previously been shown to vary widely in their interactions with canine serum proteins and also in their distributions between chloroform and water (Schach von Wittenau and Yeary, 1963). The latter characteristic was taken as an indication of lipid solubility. The values are listed in table 1.

METHODS. Standard bioassays suitably modified for the new antibiotics were used for the determination of drug concentrations (Grove and Randall, 1955).

Efforts to detect antibacterial substances other than the administered drugs in the body fluids of the animals failed. The techniques used employed chromatography and bioautography, similar to those described by Lees *et al.* (1961). Analogous results with tetracycline antibiotics were obtained by other investigators (Kelly and Buyske, 1960; Kelly *et al.*, 1961; Eisner and Wulf, 1963). Thus, the bioassays determined unchanged drug only.

The drugs were administered as solutions by stomach tube to 4 dogs every 6 hr in the indicated (table 2) quantities. The animals were dosed five times at 6-hr intervals and sacrificed 6 hr after the last dose. The drugs were administered in different quantities in order to



	R_1	R_2	R_3	R_4
6-METHYLENEOXYTETRACYCLINE (MOTC)	H	CH_2		OH
OXYTETRACYCLINE (OTC)	H	CH_3	OH	OH
TETRACYCLINE (TC)	H	CH_3	OH	H
a-6-DEOXYOXYTETRACYCLINE (DOOTC)	H	CH_3	H	OH
6-DEMETHYLCHLORTETRACYCLINE (DMCT)	CL	H	OH	H
6-DEMETHYL-6-DEOXYTETRACYCLINE (DMDOTC)	H	H	H	H

FIG. 1. Structures of tetracycline antibiotics.

minimize differences originating from variations in oral absorption efficiency. Since oral absorption of a compound frequently is a function of lipid solubility (Brodie and Hogben, 1957), the most lipid-soluble drug was administered in the smallest quantity, while the highest dose was employed for the least lipid-soluble compound. By this method, comparable total drug quantities in the animals were achieved.

The tissues were extracted by adding to a 5-g sample a cold solution (20 ml) consisting of 7 parts dimethylformamide, 5 parts water and 1 part concentrated hydrochloric acid. The resulting mixture was homogenized for 5 min in a Waring Blendor. The homogenate then was shaken mechanically for 30 min and subsequently centrifuged (1000 × *g*) for 20 min. After determination of the total weight of supernatant and sediment, the supernatant was assayed for drug content after buffering to pH 4.5. The value obtained permitted the calculation of drug concentrations in the tissue sample. For the extraction of OTC, the extracting solution was modified to consist of 6 parts dimethylformamide, 12.5 parts water and 1.5 parts concentrated hydrochloric acid.

Five samples of each tissue were assayed and the results were averaged. Corrections were made according to the recoveries obtained from control homogenates. For the controls, drug was added to various tissue homogenates of an untreated animal and the recovery of drug was determined. Between 20 and 40 recoveries were attempted for each drug. The results did not indicate that recovery from one tissue was more efficient than from another. The recoveries were: for OTC, 78%, S.D. 22%; for MOTC, 80%, S.D. 16%; for DMCT, 93%, S.D. 17%; for DOOTC, 87%, S.D. 16%; and for DMDOTC, 73%, S.D. 11%.

TABLE 1

Canine serum protein binding and distribution coefficients between chloroform and aqueous phosphate buffer of tetracyclines[a]

Compound	Canine Serum Protein Binding	K[$CHCl_3/H_2O$ (pH 7.4)] × 10^3
	%	
OTC	27	7.2
MOTC	94	72
DMCT	75	72
TC	80	95
DOOTC	82	475
DMDOTC	92	6250

[a] Schach von Wittenau and Yeary, 1963.

The free drug concentrations in serum were calculated from serum protein-binding data and the measured total drug concentrations in serum (Schach von Wittenau and Yeary, 1963).

RESULTS AND DISCUSSION. An inspection of the data listed in table 2 leads to the conclu-

TABLE 2

Drug concentrations (μg/g) after repeated oral administration in serum and tissues of dogs 6 hr after the 5th dose

Drug	Dose	Serum		Tissues					
		27 hr	30 hr	Heart	Lung	Muscle (femoral)	Muscle (intercostal)	Liver	Kidney
	mg/kg								
OTC	5 × 25	4.5 ± 0.6	4.3 ± 1	5.0 ± 1	4.4 ± 0.7	5.1 ± 1.1	4.0 ± 0.3	9.6 ± 3	11.5 ± 5
MOTC	5 × 15	9.8 ± 3.7	10.6 ± 2.9	4.3 ± 1.2	3.5 ± 1.1	3.1 ± 0.8	2.3 ± 0.7	7.3 ± 3.1	10.0 ± 2.6
DMCT	5 × 15	2.4 ± 0.9	2.2 ± 0.8	3.3 ± 0.7	2.4 ± 0.4	2.9 ± 0.7	2.4 ± 0.3	7.7 ± 3.5	11.7 ± 2.8
DOOTC	5 × 7.5	6.2 ± 2.2	6.5 ± 1.7	13.1 ± 6.1	8.6 ± 3.9	9.1 ± 3.8	8.3 ± 3.9	18.0 ± 7.5	24.8 ± 13
DMDOTC	5 × 4.5	5.5 ± 1.3	6.0 ± 1.1	8.6 ± 0.2	6.6 ± 0.2	5.5 ± 1.5	4.7 ± 0.9	9.4 ± 0.3	22.7 ± 10

The animals were dosed at 0, 6, 12, 18 and 24 hr, and sacrificed at 30 hr. Serum was also collected 3 hr after the last dose. The data were obtained from 4 dogs each, and are expressed as mean values ± the standard deviation.

sion that in comparing different but related compounds little can be inferred concerning tissue penetration from drug serum concentrations alone. Although both compounds are present in tissue in similar quantities, MOTC shows a much higher serum concentration than DMCT. The latter phenomenon is observed also after single intravenous administration of equal quantities (Schach von Wittenau and Yeary, 1963), and is mainly due to a difference in canine serum protein binding of these drugs. If allowance is made for serum protein interactions and the free drug concentrations in serum are calculated, MOTC and DMCT resemble each other closely. The free drug concentrations as well as the calculated ratios of (drug tissue concentrations)/(calculated free drug serum concentrations) are similar (table 3).

At the time the animals were sacrificed, 6 hr after the 5th dose, OTC shows a drug serum concentration intermediate between MOTC and DMCT, but, because of its relatively low serum protein binding, a calculated free drug concentration much higher than that of the others. Correspondingly, the ratios of (OTC in tissues)/(unbound OTC in serum) are much lower. It will be noted that while MOTC and DMCT are similar in their lipid solubilities, OTC is considerably less lipid-soluble.

A comparison of all data permits the generalization for the tetracyclines discussed here, that the ratio of total drug in tissues to calculated free drug in serum increases with increasing lipid solubility. To illustrate this correlation, the ratios for cardiac and skeletal muscle tissues are depicted *vs.* log K($CHCl_3$/H_2O) in figure 2. The proportions for other tissues correlated qualitatively in a similar manner. Liver and kidney, however, show great variations, which is not surprising in view of their special functions in the elimination of the drugs.

Calculations based on data reported by

TABLE 3

Calculated free drug concentrations in serum 6 hr after the 5th oral dose and calculated ratios of (total drug in tissue)/(calculated free drug in serum)

Drug	Dose	Calculated Free Drug Concentration in Serum	(Drug Concentration in Tissue)/(Calculated Free Drug Concentration)					
			Heart	Lung	Muscle (femoral)	Muscle (intercostal)	Liver	Kidney
	mg/kg	*μg/ml*						
OTC	5 × 25	3.1 ± 0.8	1.6 ± 0.08	1.5 ± 0.2	1.7 ± 0.4	1.3 ± 0.3	3.1 ± 0.7	3.7 ± 0.5
MOTC	5 × 15	0.64 ± 0.17	6.7 ± 0.4	5.8 ± 0.7	4.9 ± 0.5	3.7 ± 1.1	11.3 ± 1.6	15.8 ± 2.7
DMCT	5 × 15	0.54 ± 0.19	6.8 ± 2.6	4.8 ± 1.5	5.6 ± 1.3	4.9 ± 1.9	15.9 ± 9.5	23.8 ± 10.4
DOOTC	5 × 7.5	1.16 ± 0.30	10.8 ± 2.6	7.3 ± 2.0	7.6 ± 1.4	6.9 ± 1.7	15.2 ± 4.0	20.5 ± 6.5
DMDOTC	5 × 4.5	0.47 ± 0.08	18.6 ± 2.8	14.3 ± 2.2	11.8 ± 4.0	10.0 ± 2.2	20.5 ± 3.9	47.0 ± 14.5

The drugs are listed in the order of increasing lipid solubility. The data were obtained from 4 dogs each, and are expressed as mean values ± standard deviation.

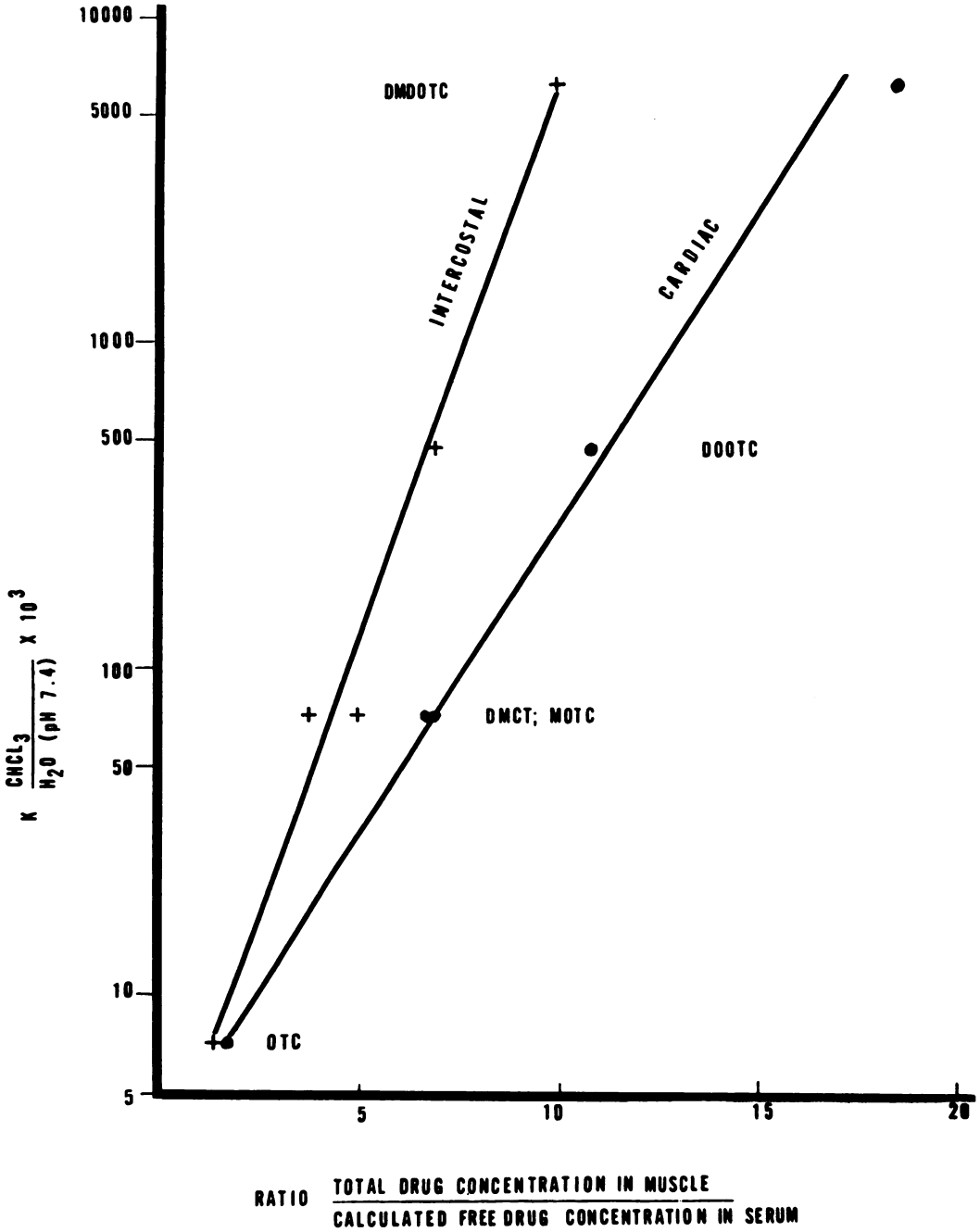


FIG. 2. Means of the ratios (total drug concentration)/(calculated free drug concentration in serum) (table 3) are plotted against the partition coefficients [$CHCl_3/H_2O$ (pH 7.4)] $\times 10^3$ (table 1) for muscle and heart tissues.

Kelly *et al.* (1961), who determined tissue concentrations of DMCT in dogs 4 hr after an intravenous dose when the drug plasma concentrations were higher than those reported in this study, suggest that these ratios prevail under a wide variety of conditions. Additional support for this view is obtained from the work of Kohn *et al.* (1960), who compared, in dogs,

DMCT and tetracycline (TC) plasma concentrations which were maintained at a relatively constant level by drug infusion, with corresponding tissue concentrations. The ratios for DMCT again are similar to those listed in table 3 and, interestingly, the values for TC are like those expected if its lipid solubility and canine serum protein binding (table 1) are taken into consideration.

Another point of interest is that drug serum concentrations as determined in dogs are of value for predicting drug serum concentrations in humans if the serum binding data are known. This is a consequence of the similarity of the drug distribution processes in the two species between the three postulated compartments: tissues, interstitial fluid and serum. The antibiotic concentration in interstitial fluid is largely determined by the equilibrium of the drug between tissues and interstitial fluid, since a relatively small percentage of an administered dose is bound to serum proteins even if serum protein binding is high. This equilibrium would be expected to differ little between species, although it is perhaps different when only one organ or tissue is being considered. Differences in serum protein binding between species then determine whether drug serum concentrations are different for different species.

To illustrate this point, the example of MOTC will be cited. Intravenous administration of 10 mg/kg to dogs (Schach von Wittenau and Yeary, 1963) yielded a drug serum concentration-time curve that showed by extrapolation a 0-hr value of about 40 μg/ml. Considering serum protein binding (table 1), this corresponds to a free drug concentration[1] of about 2.4 μg/ml. The same free drug concentration is expected to be present in humans if a total of 10 mg drug per kg b.wt. is present in the whole body. Since the binding of MOTC to human serum protein is about 80% (Schach von Wittenau and Yeary, 1963), a total drug serum concentration of about 12 μg/ml is expected.

Data obtained from the literature are collected in table 4. They show that after an equal intravenous dose of a particular tetracycline the free drug concentrations achieved in humans and dogs are similar, while the total drug serum concentrations vary according to the serum protein binding typical for each species.

TABLE 4

Drug serum concentrations and free drug concentrations (μg/ml) in humans and dogs after intravenous administration of tetracyclines

The data are taken from the literature. The data for humans are corrected: *i.e.*, when 500 mg/person were administered, the serum concentration was multiplied by a factor of 1.4; when 250 mg/person were given, the factor was 2.8. The data thus are compared on the basis of 10 mg/kg, the doses given to dogs. All data are extrapolated to 0 time from the second phase of the drug serum concentration-time curves.

Drug	Human		Dog	
	Drug serum concentration	Calculated free drug concentration	Drug serum concentration	Calculated free drug concentration
OTC	11[a]	7.5	10[b]	7
TC	7.4[c]	3.3	12.3[d]	2.5
MOTC	10[c]	2	40[b]	2.4
DMCT	8.5[e]	2.1	8[b]	2

[a] Dimmling and Nowicki (1965).
[b] Schach von Wittenau and Yeary (1963).
[c] Kunin (1962).
[d] Pindell *et al.* (1959).
[e] Kunin *et al.* (1959).

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[1] The free drug concentration in interstitial fluid is difficult to determine, but should be similar to the measurable free drug concentration in serum, a relation which has been shown to be true for penicillins (Scholtan and Schmid, 1962; Verwey and Williams, 1962).

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