Comparison of hematocrits showed that average values obtained following the delayed-release capsule (32.4%) and following ferrous sulfate (31.1%) were statistically significant ($p < 0.05$). This finding indicated that the bioavailability of iron from the delayed-release capsule was equivalent to, and perhaps somewhat better than, ferrous sulfate solution when using hematocrit regeneration as an index of bioavailability. A difference of 1.2 hematocrit units (only 3.6% of the average hematocrit) would have been statistically significant. The careful selection of a dose of iron that was below the maximum capable of being absorbed by the gut and the regenerating capacity of the bone marrow was probably an important reason for the success of this approach to iron bioavailability methodology. The method demonstrated that delaying the release of iron in the pelletized iron preparations tested in no way impaired its bioavailability.

REFERENCES

(1) R. W. Beal, *Drugs*, **2**, 190 (1971).

(2) L. Hallberg, H. Brise, and L. Solwell, *Proc. VII Cong., Int. Soc. Hematol.*, **2**, 524 (1958).

(3) H. Brise and L. Hallberg, *Acta Med. Scand.*, **171**, 1, (1962).

(4) G. Matrone, E. L. Thomason, and C. R. Bunn, *J. Nutr.*, **72**, 459 (1960).

(5) J. Hannan, *Vet. Rec.*, **88**, 181 (1971).

(6) K. Furugouri, *J. Animal Sci.*, **34**, 421 (1972).

(7) E. Kolb, *Adv. Vet. Sci.*, **8**, 49 (1963).

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[x] To whom inquiries should be directed.

Antibacterial Structure–Activity Relationships Obtained with Resistant Microorganisms I: Inhibition of R-Factor Resistant *Escherichia coli* by Tetracyclines

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Abstract □ Apparent partition coefficients and inhibitory activities against sensitive and resistant *Escherichia coli* were determined for 14 tetracyclines. The difference in the kinetics of inhibition of the two organisms is discussed in terms of their permeabilities. The partition coefficients were determined in an octanol–buffer system. Values for eight compounds were in general agreement with the literature; values for the remaining six compounds had not been reported previously. Growth of the organisms was determined by a single-point turbidimetric method in the presence and absence of tetracyclines. Inhibitory activities were obtained by a kinetic treatment. Derived rate constants for the sensitive organism were linearly related to antibiotic concentration. For the resistant organism and 12 compounds, the derived rate constants and antibiotic concentration were related in a manner typical of saturation kinetics. These inhibitory activities were related to the partition coefficients, while activities against the sensitive strain were not. These findings suggest that activity against the resistant strain is permeability controlled but that activity against the sensitive strain has a different rate-determining step.

Keyphrases □ Antibacterial activity—various tetracyclines, related to partition coefficients, resistant and sensitive microorganisms, structure–activity relationships □ Tetracyclines, various—antibacterial activity, related to partition coefficients, resistant and sensitive microorganisms, structure–activity relationships □ Partition coefficients—various tetracyclines, related to antibacterial activity, resistant and sensitive microorganisms □ *Escherichia coli*—resistant and sensitive, inhibition by various tetracyclines, related to partition coefficients, structure–activity relationships □ Structure–activity relationships—various tetracyclines, antibacterial activity, resistant and sensitive microorganisms

The development of safe, effective antimicrobial drugs has revolutionized medicine in the last 30 years. Unfortunately, microorganisms are highly versatile, and the brilliance of the chemotherapeutic achievement has been dimmed by the emergence of microbial strains resistant to these drugs. This emergence of resistant pathogens is becoming an increasingly important problem. In addition to limiting the use of antibiotics, two possible approaches to the problem would be to seek entirely new antibacterial agents and to modify existing chemotherapeutic agents. The latter approach has the advantage that derivatives of present chemotherapeutic agents are likely to retain the high benefit to risk ratios of the parent drugs.

The general mechanisms by which microorganisms may become resistant to antibiotics were summarized (1), and the one where drug modification might effectively overcome drug resistance is the loss of cell permeability to the drug. Changes in the physicochemical nature of a drug resulting in decreased or increased cell permeabilities were described for many systems (2).

Davies (3) stated that, as far as can be ascertained, all tetracycline-resistant strains obtained from clinical situations are resistant because they carry an R-factor. The nature of R-factor resistance to tetracyclines was investigated extensively and reviewed (4, 5). These studies indicate that tetracycline resistance is related to a decreased uptake of tetracyclines by resistant cells. Several investigators showed that cell-free protein-synthesizing systems from sensitive and resistant strains were equally inhibited by tetracyclines (6–8).

Escherichia coli accumulated oxytetracycline at high concentrations (9), and this accumulation was inhibited by azide and 2,4-dinitrophenol and was dependent upon the energy source in the medium. Similar observations

Table I—Partition Coefficients and Inhibitory Activities against Sensitive *E. coli* W (K_{sens}) and Resistant *E. coli* (K_R+)

Tetracycline	Partition Coefficients		Inhibitory Activities	
	Experimental	Lit. (22)	$K_{sens} \times 10^6$	$K_R + \times 10^6$
5a(6)-Anhydro-	6.0	—	0.12	0.108[a]
4-Dedimethylamino-	4.95	5.79	0.077	0.0208[a]
7-Dimethylamino-6-demethyl-6-deoxy-	1.47	1.48	0.25	0.6452
6-Demethyl-6-deoxy-	0.99	0.96	0.25	0.0714
9-Dimethylamino-6-demethyl-6-deoxy-	0.82	0.77	0.076	0.0222
7-Nitro-6-demethyl-6-deoxy-	0.78	—	1.45	0.0476
7-Chloro-	0.37	0.32	0.95	0.0125
7-Chloro-6-demethyl-	0.25	0.19	1.10	0.0057
9-Amino-6-demethyl-6-deoxy-	0.21	—	0.35	0.025
9-Nitro-6-demethyl-6-deoxy-	0.14	—	0.145	0.0030
5-Hydroxy-	0.12	0.087	0.65	0.0011
7-Amino-6-demethyl-6-deoxy-	0.09	—	0.19	0.0018
Unsubstituted	0.09	0.052	0.60	0.0018
5a(11)-Dehydro-7-chloro-	0.057	—	—	—[a]
7-Bromo-6-demethyl-6-deoxy-[b]	—	—	0.07	0.105
12a-Deoxy-[b]	—	—	0.0085	0.015[a]
4-Methiodide	—	0.01	0.0073	—

[a] These compounds had a linear relationship between tetracycline concentration and derived rate constants for growth of the resistant strain. [b] Samples of these compounds varied in the observed partition coefficients obtained at different wavelengths, so values for these compounds have not been used.

were reported with tetracycline and chlortetracycline at low concentrations (7). The resistance was associated with a marked decrease in the ability of *E. coli* to accumulate tetracyclines (7, 10–12). Similarly, Sompolinsky *et al.* (13) found a decreased uptake in resistant staphylococci, and Tseng and Bryan (14) noted decreased uptake of tetracycline in resistant *Pseudomonas* strains. The uptake of tetracycline by sensitive and resistant *E. coli* was correlated to the minimum inhibitory concentrations of the strains studied (15).

The exact mechanism of the uptake of tetracyclines by sensitive cells is not yet established. It was suggested that accumulation in *Staphylococcus aureus* follows saturation kinetics initially but that a multiplicity of sites becomes available at high antibiotic concentrations and antibiotic concentration becomes limiting (16). The uptake of tetracyclines by *E. coli* as a function of tetracycline concentration was reported to be biphasic (17). Resistance was suggested to be associated with a requirement for higher antibiotic concentrations before the second mode of accumulation became operative. However, Reynard and Nellis (18) were unable to find the initial phase of saturation kinetics in *E. coli*.

Regardless of the exact mechanism, tetracycline resistance probably is associated with a reduced ability to accumulate the antibiotic within the resistant cells. If this assumption is true, then alteration of the balance of water–lipid solubility of the antibiotics might lead to improved permeation by passive diffusion. As lipophilicity increased in a series of tetracycline derivatives, their activity against a tetracycline-resistant *S. aureus* increased (19). Furthermore, recent activity determinations (20) using various resistant *Staphylococcus* strains were also in general agreement with this principle. The present work extends these findings to R-factor resistant *E. coli* in a quantitative fashion.

EXPERIMENTAL

Materials—A tetracycline-sensitive *Escherichia coli* W (ATCC 9637) and a tetracycline-resistant *Escherichia coli* (ATCC 19215, recipient of an R-factor conferring multiple resistance to tetracycline, chloramphenicol, streptomycin, and sulfonamides) were used as test organisms. A high level of tetracycline resistance was induced in the R-factor strain by subculturing it in the presence of tetracycline (12). The culture broth was a high peptone medium buffered at pH 6.65 with potassium salts of phosphoric acid as previously described (21).

Samples of tetracycline, oxytetracycline, and chlortetracycline were purchased[1]; the remaining derivatives were supplied by the manufacturers[2]. Apparent partition coefficients were determined between a pH 6.6 phosphate buffer and 1-octanol[3]. Buffer solutions were prepared with the potassium salts of phosphoric acid and adjusted to an ionic strength of 0.1 *M* with sodium chloride. The water used in these solutions had been redistilled from an all-glass still[4].

Determination of Apparent Partition Coefficients of Tetracycline Analogs—A solution of the tetracycline under study was prepared in buffer saturated with octanol (buffer phase). Sufficient quantities of this solution were placed in 50-ml glass-stoppered centrifuge tubes so that the final concentration was 0.5 m*M* when the volume of solution was brought to 10 ml by the addition of buffer phase. Exactly 10 ml of octanol saturated with buffer (octanol phase) was added to each tube. Duplicate tubes were prepared for each tetracycline analog studied as well as blank tubes containing only buffer phase and octanol phase.

The capped tubes were mounted horizontally in a shaker[5] and vigorously shaken for 10 min. After centrifuging at 1500 rpm for 20 min, a sample of the buffer phase was carefully removed from each tube with a syringe equipped with a 15.2-cm (6-in.) 20-gauge needle. Aliquots of these samples as well as aliquots of the original tetracycline analog solutions were diluted with buffer phase and assayed spectrophotometrically[6]. Absorption measurements were made at wavelengths corresponding to the tetracycline peaks in the regions of 275 and 350 nm and at maxima and/or minima at about 250 and/or 450 nm, if they existed for the derivative. After correcting for blank readings, the absorbance values obtained at each wavelength for each sample and the absorbance values for its corresponding original solution were used to calculate the concentrations of tetracycline analog in both the buffer and octanol phases.

The apparent partition coefficients reported are average values obtained at all wavelengths studied (Table I). The agreement of partition coefficients obtained at several wavelengths for the tetracycline analogs is taken as an indication of their high degree of purity. Accordingly, the values obtained for two of the analogs studied are not reported since the apparent partition coefficients varied as a function of wavelength.

Inhibitory Activity Determinations with Tetracycline-Sensitive *E. coli* W—The inhibitory activities with the sensitive *E. coli* W were determined for tetracycline and its 15 analogs according to the following

[1] Calbiochem, La Jolla, CA 92037.

[2] The authors are grateful to Dr. James H. Boothe, Lederle Laboratories, Pearl River, N.Y., and Dr. Charles R. Stephens, Pfizer Laboratories, Groton, Conn., for assistance in providing the tetracycline analogs.

[3] Certified reagent grade, Fisher Scientific Co., Pittsburgh, PA 15238.

[4] Model AG-1a, Corning Glass Works, Corning, NY 14803.

[5] Dubnoff, Precision Scientific Co., Chicago, Ill.

[6] Gilford Instrument Laboratories, Oberlin, OH 44074.

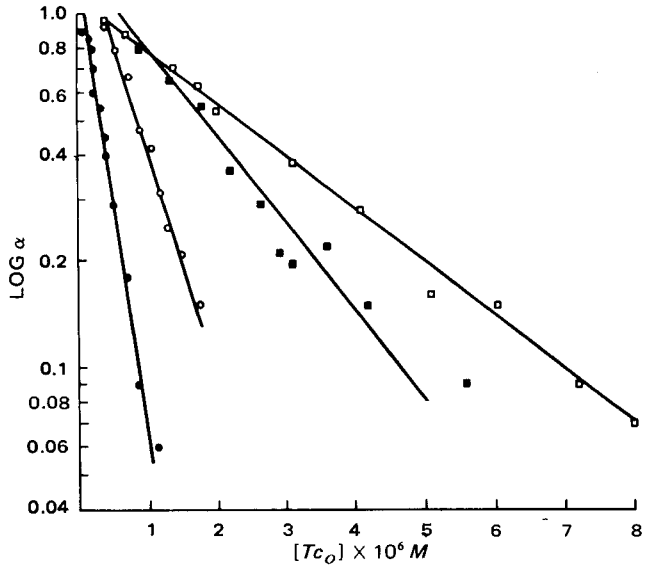

Figure 1—*Fractional activity against tetracycline sensitive* E. coli *W as a function of the concentration of tetracycline analogs. Key:* ●, *7-nitro-6-demethyl-6-deoxytetracycline;* ○, *oxytetracycline;* ■, *6-demethyl-6-deoxytetracycline; and* □, *9-nitro-6-demethyl-6-deoxytetracycline.*

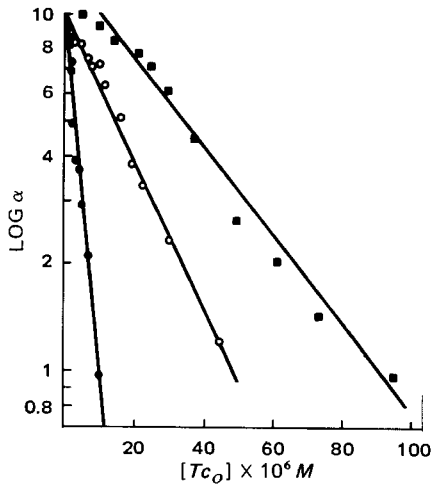

Figure 2—*Fractional activity against tetracycline-resistant* E. coli R^+ *as a function of the concentration of tetracycline analogs. Key:* ●, *5a(6)-anhydrotetracycline;* ○, *4-dedimethylaminotetracycline; and* ■, *12a-deoxytetracycline.*

procedure. Aliquots of a tetracycline analog solution were added to 3 ml of broth in sterile 6-ml capped tubes[7] so that a range of 12 tetracycline concentrations was obtained. Duplicate tubes were prepared and run for all analog concentrations. An overnight peptone broth culture of *E. coli* W was diluted with fresh broth so that a concentration of 1×10^7 *E. coli* W/ml was obtained when an aliquot (5, 10, or 20 μl) was added to each of the 24 tubes containing tetracycline and to two tubes without tetracycline.

The tubes were incubated for 3.5 hr at 37.5° in a constant-temperature water bath. Growth was stopped by the addition of 20 μl of formaldehyde solution. The turbidity of the cultures was determined[6] at 500 nm. The fractional activity, α, was calculated from the ratio of the turbidity of a culture grown in the presence of the tetracycline analog to that obtained for cultures without antibiotic. If one assumes that the turbidity of the cultures is proportional to the number of organisms per milliliter of culture and that growth of the cultures in the presence and absence of tetracyclines is first order in organisms per milliliter (21), then the logarithm of α is proportional to the difference in the growth rate constants in the presence (k_{app}) and absence (k_0) of antibiotic.

Inhibitory Activity Determinations with Tetracycline-Resistant *E. coli*—Inhibitory activities with the resistant *E. coli* were determined for tetracycline and its 14 analogs by the same procedure as that outlined for the sensitive organism except: (*a*) the overnight culture was 320 μ*M* in tetracycline, (*b*) the volume of the overnight culture added to the experimental cultures was between 100 and 300 μl, and (*c*) the time of incubation was 5 hr instead of 3.5 hr.

RESULTS AND DISCUSSION

Apparent Partition Coefficients of Tetracycline Analogs—Colaizzi and Klink (22) reported the apparent partition coefficients of a number of tetracycline analogs at several pH values, including 6.6. Their work was extended by determining the apparent partition coefficients of six compounds not available to those authors. In addition, the values reported by Colaizzi and Klink were verified for eight tetracycline analogs (Table I). The most lipophilic tetracycline analog studied was 5a(6)-anhydrotetracycline, with an apparent partition coefficient (K_p) of 6.0; the most hydrophilic studied was 5a(11)-dehydro-7-chlorotetracycline (K_p = 0.057). Agreement with reported values (22) was excellent except for the most hydrophilic derivatives (tetracycline and oxytetracycline), where the experimental error involved might be expected to be highest for procedures in which only the aqueous phase of the biphasic system is assayed.

As already mentioned, the agreement of apparent partition coefficients calculated at different wavelengths was excellent for the tetracyclines reported, indicating a high degree of purity. Two samples of 7-bromo-6-demethyl-6-deoxytetracycline were examined and showed considerable variation in the apparent partition coefficients calculated at the different wavelengths. Values for this compound as well as a similarly behaving sample of 12a-deoxytetracycline are not reported. The biological activity of these samples (Table I) is, however, similar to that reported (19).

Inhibitory Activities of Tetracycline Analogs against Sensitive *E. coli*—Initial estimates of the activities of the derivatives were made by plotting fractional activities, α, *versus* antibiotic concentration and estimating the concentration of antibiotic that would cause a 50% inhibition of growth. However, it became apparent that plots of log α *versus* antibiotic concentration were linear for all of the compounds studied with the tetracycline-sensitive organism, *E. coli* W (Fig. 1). This finding can be explained if it is assumed that the turbidity of the cultures is proportional to the number of cells per milliliter of culture (N) and that growth is first order for the period of incubation in N, both in the presence ($N = N_0 e^{k_{app} t}$) and absence ($N = N_0 e^{k_0 t}$) of antibiotic. Since N_0 is the same for both cultures, log α becomes equal to $2.3t(k_{app} - k_0)$. It was shown previously that for tetracycline inhibiting *E. coli* B/r (23) and for tetracycline analogs inhibiting *E. coli* W (21):

$$k_{app} = k_0 - K(Tc) \qquad \text{(Eq. 1)}$$

where (Tc) is the concentration of tetracycline analog, and K is a constant typical of the tetracycline analog. Rearrangement of Eq. 1 shows that a plot of log α *versus* (Tc) should be linear. The data suggest that these assumptions are true for all tetracycline derivatives studied with *E. coli* W. The negative slopes of such plots (Fig. 1) were evaluated, and the values obtained for each analog are listed as K_{sens} values in Table I. These activities were in substantial agreement with those previously published for these compounds and this organism but obtained by the more rigorous methods of bacterial growth kinetics (21). This agreement is shown by:

$$K_{sens} = -9.347 + 482.1\, K^I_{avg} \qquad r = 0.98\ n = 15 \qquad \text{(Eq. 2)}$$

where K^I_{avg} is the K of Eq. 1 obtained from kinetic measurements of cell division (total and viable), nucleic acid synthesis, and protein synthesis.

Inhibitory Activities of Tetracycline Analogs against Resistant *E. coli*—The *E. coli* employed was resistant to tetracycline to the extent that the concentration of tetracycline causing a 50% inhibition in growth in the test system was about 175 times greater than that necessary to inhibit *E. coli* W. In contrast to the results already described for *E. coli* W, plots of log α *versus* (Tc) were only linear for four tetracycline analogs (Fig. 2). In addition to the three compounds shown on this figure, 5a(11)-dehydro-7-chlorotetracycline also exhibited a linear relationship between log α and tetracycline concentration. Because of their extremely weak activity, it was not possible to determine the relationship between log α and tetracycline concentration for isochlortetracycline and tetracycline methiodide over a wide range of inhibition, but preliminary experiments indicated that they might also have a linear relationship. All

[7] Falcon, Oxnard, CA 93030.

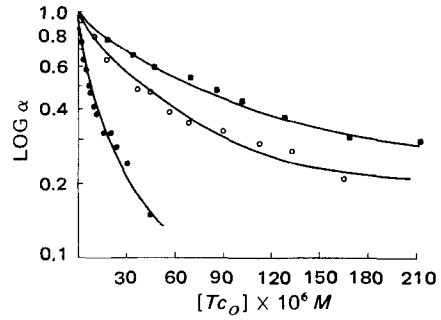

Figure 3—*Fractional activity against tetracycline-resistant* E. coli R^+ *as a function of the concentration of tetracycline analogs. Key:* ●, *6-demethyl-6-deoxytetracycline;* ○, *chlortetracycline; and* ■, *demeclocycline.*

of these compounds are unusual tetracyclines in that either their phenyl diketone moiety or their 4-dimethylamino moiety is modified. These moieties are usually considered to be essential for tetracycline-like activity. However, the activities of these compounds against sensitive *E. coli* W are correlatable with their physicochemical properties (24).

Plots of log α *versus* tetracycline concentration for the remaining 12 compounds studied were not linear, as shown for three of the compounds in Fig. 3. The curvature of these plots suggested that a data treatment typical of saturation kinetics would be useful. Plots of $-(1/\log \alpha)$ *versus* $1/(Tc)$ and of $-[(Tc)/\log \alpha]$ *versus* (Tc) were linear for these compounds. These plots would be expected to be linear if the assumptions given that allow log α to be related to $k_{app} - k_0$ are true and if the more complicated expression:

$$k_{app} = k_0 - K_a(Tc)/1 + K_b(Tc) \qquad \text{(Eq. 3)}$$

is applicable instead of Eq. 1. Such an expression has not been reported as being applicable to tetracycline activities, but similar expressions are known to apply to other antibacterial agents such as sulfonamides (25, 26), trimethoprim (26), and erythromycin (27) and to certain phases of lincomycin inhibition (27). Examples of the fit of the data to Eq. 3 are shown in Figs. 4 and 5. In general, the linearity of these lines is excellent considering the assumptions in the data treatment. Inhibitory activities, K_{R^+}, derived from the slopes and intercepts of the plots are given in Table I.

As might be expected, the activities against the sensitive strain were not related to the activities obtained with the resistant strain, as shown by:

$$K_{R^+} = 0.96 - 0.057\,K_{sens} \qquad r = -0.15 \quad n = 15 \qquad \text{(Eq. 4)}$$

Structure–activity relationships using activities obtained with sensitive strains (28, 29) would not be expected to be valid for resistant strains. Blackwood and English (19) suggested a qualitative relationship between activities obtained against a resistant *Staph. aureus* and lipophilicity. Our data show a quantitative relationship between tetracycline activity against the resistant *E. coli* and lipophilicity, as given in Eq. 5 and shown graphically in Fig. 6:

$$\log K_{R^+} = 1.98 + 1.74 \log K_p \qquad r = 0.918 \quad n = 11 \quad F_9^1 = 48.5 \qquad \text{(Eq. 5)}$$

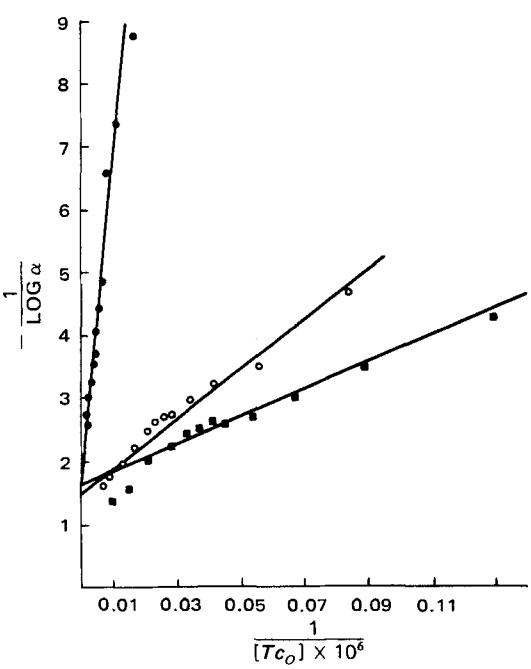

Figure 4—*Relationship between fractional activity against tetracycline-resistant* E. coli R^+ *and the concentration of tetracycline analogs. Key:* ●, *7-amino-6-demethyl-6-deoxytetracycline;* ○, *9-amino-6-demethyl-6-deoxytetracycline; and* ■, *7-nitro-6-demethyl-6-deoxytetracycline.*

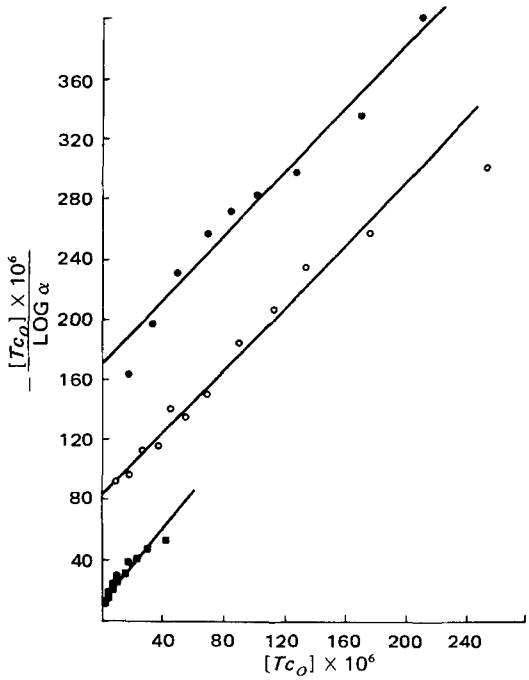

Figure 5—*Relationship between fractional activity against tetracycline-resistant* E. coli R^+ *and the concentration of tetracycline analogs. Key:* ●, *6-demethyl-6-deoxytetracycline;* ○, *chlortetracycline; and* ■, *demeclocycline.*

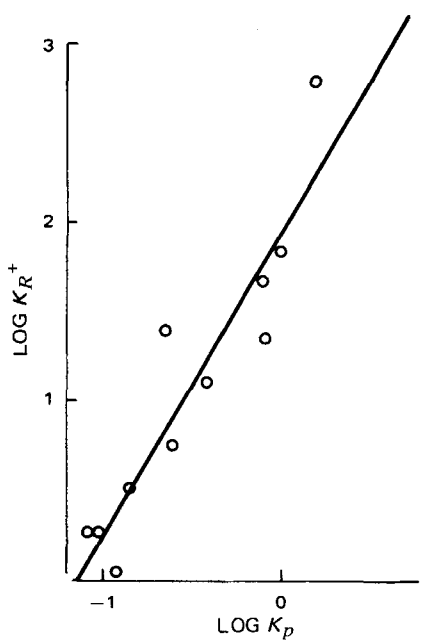

Figure 6—*Relationship of inhibitory activities against tetracycline-resistant* E. coli R^+ *and partition coefficients of tetracycline analogs.*

Inclusion of higher order terms in log K_p did not improve the fit of the data, nor did such expressions allow the fit of the compounds that were typified by a linear relationship between log α and tetracycline concentration. In addition, inclusion of several reported quantum mechanical parameters (24) for tetracyclines did not improve the fit of the data. The activities of the tetracycline antibiotics with intact phenyl diketone and 4-dimethylamino moieties against resistant *E. coli* probably are directly related to their lipophilicity. The compound that had the highest partition coefficient and the highest activity of those studied was 7-dimethylamino-6-demethyl-6-deoxytetracycline (minocycline).

Model of Tetracycline Inhibitory Activity—A reasonable explanation of the discussed structure–activity relationships may be derived from the model given by Garrett (30) or Peradejordi *et al.* (24). In this model, tetracycline outside the cell (Tc_o) is considered to permeate the cell wall (Tc_i) and then to react with a receptor reversibly in such a manner that growth is inhibited according to Schemes I and II:

$$Tc_o \underset{}{\overset{K_1}{\rightleftharpoons}} Tc_i$$

Scheme I

$$Tc_i + \text{receptor} \overset{K_2}{\rightleftharpoons} Tc_i\text{–receptor}$$

Scheme II

If one assumes that the generation of *E. coli* is related to the fraction of free receptors as given by:

$$dN/dt = k_o(1 - \theta)N \qquad \text{(Eq. 6)}$$

where θ is the fraction of receptors occupied by tetracyline, then Schemes I and II and Eq. 6 may be rearranged and related to the experimentally observed growth rate constant, k_{app}, as follows:

$$k_{app} - k_o = -\frac{k_o K_1 K_2 Tc_o}{1 + K_1 K_2 Tc_o} \qquad \text{(Eq. 7)}$$

Equation 7 is similar to Eq. 3, but K_a is now considered to be $k_o K_1 K_2$ and K_b is $K_1 K_2$.

The linear relationship between log α and tetracycline concentration found for *E. coli* W and for four tetracyclines with the resistant *E. coli* results when $K_1 K_2 Tc$ is less than 1. Under these conditions, the K_{sens} values reported are equal to $k_o K_1 K_2$.

In contrast, the complete expression is necessary to fit the data obtained for the 12 tetracyclines with normal phenyl diketone and 4-dimethylamino moieties against the resistant *E. coli*. The reported inhibitory activity, K_{R+}, would be equal to $K_1 K_2$ if this simple model is correct. Since the two activities are not related (Eq. 4) and since K_{R+} is related to the partition coefficients (Eq. 5), it seems logical that K_{sens} is controlled by K_2 and that K_{R+} is controlled by K_1. Thus, the resistance to tetracyclines is associated with a change in the rate-determining step from one involving the receptor to one involving permeation into the cell, consistent with previously published theories of tetracycline resistance (3).

The four compounds with abnormal phenyl diketone or 4-dimethylamino moieties are compounds with intrinsically low activities and, in two cases, very high apparent partition coefficients. Thus, for these compounds, K_2 continues to be the rate-controlling step in the inhibition of resistant *E. coli*.

REFERENCES

(1) B. D. Davis and W. K. Maas, *Proc. Nat. Acad. Sci.*, **38**, 775 (1952).

(2) C. Hansch, *Mol. Pharmacol.*, **5**, 333 (1969).

(3) J. Davies, *Ann. Rep. Med. Chem.*, **7**, 217 (1972).

(4) T. Watanabe, *Bacteriol. Rev.*, **27**, 87 (1963).

(5) J. S. Piton, *Ergebn. Physiol.*, **65**, 15 (1972).

(6) S. Okamoto and D. Mizuno, *J. Gen. Microbiol.*, **35**, 125 (1964).

(7) T. J. Franklin and A. Godfrey, *Biochem. J.*, **94**, 54 (1965).

(8) J. A. Last, K. Izaki, and J. F. Snell, *Can. J. Microbiol.*, **15**, 1077 (1969).

(9) K. Izaki and K. Arima, *J. Bacteriol.*, **89**, 1335 (1965).

(10) K. Izaki, K. Kiuchi, and K. Arima, *ibid.*, **91**, 628 (1966).

(11) T. J. Franklin, *Biochem. J.*, **105**, 371 (1967).

(12) T. J. Franklin and B. Higginson, *ibid.*, **116**, 287 (1970).

(13) D. Sompolinsky, Y. Zaidenzaig, R. Ziegler-Schlomowitz, and N. Abramova, *J. Gen. Microbiol.*, **62**, 351 (1970).

(14) J. T. Tseng and L. E. Bryan, *Antimicrob. Ag. Chemother.*, **3**, 638 (1973).

(15) A. M. Reynard, L. F. Nellis, and M. E. Beck, *Appl. Microbiol.*, **21**, 71 (1971).

(16) B. L. Hutchings, *Biochim. Biophys. Acta*, **174**, 734 (1969).

(17) J. R. De Zeeuw, *J. Bacteriol.*, **95**, 498 (1968).

(18) A. M. Reynard and L. F. Nellis, *Biochem. Biophys. Res. Commun.*, **48**, 1129 (1972).

(19) R. K. Blackwood and A. R. English, *Adv. Appl. Microbiol.*, **13**, 237 (1970).

(20) D. Sompolinsky and J. Krausz, *Antimicrob. Ag. Chemother.*, **4**, 237 (1973).

(21) G. H. Miller, S. A. Khalil, and A. N. Martin, *J. Pharm. Sci.*, **60**, 33 (1971).

(22) J. L. Colaizzi and P. R. Klink, *ibid.*, **58**, 1184 (1969).

(23) M. R. W. Brown and E. R. Garrett, *ibid.*, **53**, 179 (1964).

(24) F. Peradejordi, A. N. Martin, and A. Cammarata, *ibid.*, **60**, 576 (1971).

(25) E. R. Garrett and O. K. Wright, *ibid.*, **56**, 1576 (1967).

(26) J. K. Seydel, E. Wempe, G. H. Miller, and L. Miller, *Chemotherapy*, **17**, 217, (1972).

(27) E. R. Garrett, S. M. Heman-Ackah, and G. L. Perry, *J. Pharm. Sci.*, **59**, 1448 (1970).

(28) A. Cammarata, S. S. Yau, J. H. Collett, and A. N. Martin, *Mol. Pharmacol.*, **6**, 65 (1970).

(29) A. Cammarata and S. J. Yau, *J. Med. Chem.*, **13**, 94 (1970).

(30) E. R. Garrett, *Progr. Drug Res.*, **15**, 271 (1971).

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