tively charged nitrogen atom of the analog fit the binding site on the enzyme. Attempts to isolate the labeled, acetylated analog from the reaction mixture were unsuccessful. The label trailed badly in all separation systems tried.

Although the analogs acted as enzyme inhibitors, they had no effect on the sperm. Whether the analogs were excluded from the cells or entered the cell and were ineffective in altering the enzyme activity cannot be discerned from the available data. These studies confirmed previous work that showed that the analogs had no biological activity (5).

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Pharmacokinetics of Doxycycline Reabsorption

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Abstract □ Two cyclic linear compartment models are proposed to investigate the reabsorption mechanism of doxycycline. In one model, reabsorption is considered to be continuous; in the other model, it is discontinuous. The continuous model, when fitted, leads to one real and two complex conjugate eigenvalues, corresponding to a regression equation consisting of a regular exponential term and an exponentially damped trigonometric expression. In spite of the apparent oscillatory nature of this regression equation, the fitted curves show no secondary peaks or humps apparent in the data. Simulation studies indicate that it may not be possible to get response profiles showing secondary peaks or humps that are experimentally detectable with linear compartment systems with cyclic pathways and continuous transfer. The model with discontinuous cyclic transfer was more flexible in describing the discrepancies in the data and appeared to be preferable to the continuous cyclic transfer model judged by the Akaike information criterion.

Keyphrases □ Doxycycline—pharmacokinetics of reabsorption, two cyclic linear compartment models proposed □ Pharmacokinetics—doxycycline reabsorption, two cyclic linear compartment models proposed □ Models—pharmacokinetics of doxycycline reabsorption

There does not appear to be general agreement about the pharmacokinetic behavior of doxycycline in humans. Gibaldi (1) claimed that the persistence of serum doxycycline levels is due to a relatively slow absorption compounded by enterohepatic cycling, while other authors proposed that it is due to an intrinsically slow rate of elimination (2, 3). The pharmacokinetic parameters for doxycycline were calculated from the data of Fabre *et al.* (3), assuming a single-compartment model (1). The elimination rate was calculated using the data points from 35 to 72 hr after administration; these values were assumed to be true postabsorptive data (1). The mean half-life for doxycycline of 9.8 hr obtained in this way does not differ greatly from the half-life of other tetracyclines. An apparent absorption half-life of 5.3 hr was calculated by the method of residuals. It was suggested (1) that the persistence of appreciable serum concentrations of doxycycline relative to other tetracyclines is a function of absorption kinetics rather than elimination kinetics.

Using the same data, a subsequent investigator (2) calculated a substantially different absorption half-life of 50 min and a quite different elimination half-life of 22–24 hr and suggested that the persistent serum levels are primarily due to slow elimination. Schach von Witteman (2) pointed out that the low values found 72 hr after dosing are not very accurate and that the emphasis placed on this limited segment of the data is not justified. However, this investigator ignored the last two data points entirely in calculating the elimination rate constant. Inclusion of these two data points results in a considerably shorter half-life.

Although it is difficult to resolve properly the absorption and elimination kinetics from oral data alone, the data (3) show signs of secondary peaks, which support the hypothesis of reabsorption. This hypothesis is further supported by a high affinity to the bile reported for doxycycline and other tetracyclines (4). If reabsorption occurs, then the calculation of intrinsic absorption and elimination half-lives using classical pharmacokinetic approaches as discussed may not apply. It is not valid to assume a postabsorptive phase in a pharmacokinetic system with significant reabsorption.

Therefore, it is of interest to investigate the kinetic behavior of doxycycline using kinetic models that do consider the drug's reabsorption. It is also valuable to investigate whether the reabsorption occurs through a continuous process as suggested previously (4) or whether it is of a discontinuous nature, possibly related to biliary intestinal secretion that occurs primarily in the form of squirts of bile into the intestine (5). This work proposes two simple pharmacokinetic models to investigate these matters using the data of Fabre *et al.* (3).

THEORY

The two linear pharmacokinetic models proposed represent simple forms of drug reabsorption. Both models contain a cyclic disposition and do not belong to the categories of models (mammillary, catenary, and other noncyclic systems) proposed most frequently in pharmacokinetics.

Model 1—This linear model proposes first-order absorption, central

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elimination, and drug distribution into an intermediate compartment. The intermediate compartment can be considered to be either the bile or a virtual compartment representing a secreted nonabsorbable compound or complex that releases the drug to the "absorption compartment" where it is subject to reabsorption.

The equation for this model is readily derived by the Veng Pedersen approach[1] (6). Equation 12 from Ref. 6 gives:

$$x_1 = L^{-1}\sum_{j=1}^{3}\frac{|\mathbf{S}|_{1j}}{|\mathbf{S}|}[x_j(0)+\bar{f}_j] = L^{-1}\frac{|\mathbf{S}|_{13}}{|\mathbf{S}|}D_F \qquad \text{(Eq. 1)}$$

Using Heaviside's expansion theorem and $c = x_1/V$, Eq. 1 becomes:

$$c = \frac{D_F}{V}\sum_{j=1}^{3}P(\lambda_j)\exp\lambda_j(t-T_L)_+ \qquad \text{(Eq. 2)}$$

where:

$$P(s) = |\mathbf{S}(s)|_{13}/|\mathbf{S}'(s)| \qquad \text{(Eq. 3)}$$

$$|\mathbf{S}(s)| = s^3 + a_2s^2 + a_1s + a_0 = (s-\lambda_1)(s-\lambda_2)(s-\lambda_3) \qquad \text{(Eq. 4)}$$

$$|\mathbf{S}'(s)| = 3s^2 + 2a_2s + a_1 \qquad \text{(Eq. 5)}$$

$$a_2 = E_1 + E_2 + E_3 \qquad \text{(Eq. 6)}$$

$$a_1 = E_1E_2 + E_1E_3 + E_2E_3 - (k_{21}k_{12} + k_{31}k_{13} + k_{23}k_{32}) \qquad \text{(Eq. 7)}$$

$$a_0 = E_1E_2E_3 - k_{23}k_{32}E_1 - k_{21}k_{12}E_3 - k_{31}k_{13}E_2 - k_{31}k_{12}k_{23} - k_{13}k_{32}k_{21} \qquad \text{(Eq. 8)}$$

$$|\mathbf{S}|_{13} = k_{31}(s+E_2) \qquad \text{(Eq. 9)}$$

If the system shows significant reabsorption, then two of the eigenvalues of the linear system will be complex conjugate while the third will be real; otherwise, all eigenvalues will be real. In any case, the real part of any eigenvalue is negative. In the complex case, Eq. 2 can also be written:

$$c = \frac{D_F}{V}\{2[Re(P(\lambda))\cos\lambda_{Im}(t-T_L)_+ - Im[P(\lambda)]\sin\lambda_{Im}(t-T_L)_+]\exp\lambda_{Re}(t-T_L)_+ + P(\lambda_1)\exp\lambda_1(t-T_L)_+\} \qquad \text{(Eq. 10)}$$

where λ_1 is the real eigenvalue and λ_{Im} and λ_{Re} are the imaginary and real parts, respectively, of a complex eigenvalue λ satisfying $|\mathbf{S}(\lambda)| = 0$.

Although explicit formulas exist for finding the complex and/or real roots of the characteristic polynomial $|\mathbf{S}(s)|$, it is not advisable to use them because of the possibility of severe numerical instability (10). Instead, the eigenvalues were determined to the proper degree of accuracy using Laguerre's method (11) and the interactive nonlinear regression program FUNFIT (12), which was used for all curve fittings. Equation 2 was programmed to accommodate both the real and complex cases. Therefore, the microparameters and the form of the response function are not constrained but lead to a convergence to either a real or complex case, depending on the data.

Model 2—This model is identical to Model 1, except that drug transfer between Compartments 2 and 3 is not a continuous first-order process but a bolus impulse. At some time, $t = T_b$, some fraction, F_b, of the drug cumulated in the bile is spontaneously released to the intestine by a bile squirt. The equation describing this process is readily derived by standard means (6):

$$c = \left(\frac{D_F}{V}\right)\frac{k_{31}}{k_{31}+\lambda}\{\exp\lambda(t-T_L)_+ - \exp[-k_{31}(t-T_L)_+] + A[\exp\lambda(t-T_b)_+ - \exp[-k_{31}(t-T_b)_+]]\} \qquad \text{(Eq. 11)}$$

where:

$$A = \frac{F_bk_{31}k_{12}}{k_{31}+\lambda}\left\{\frac{1}{\lambda}[\exp\lambda(T_b-T_s)-1] + \frac{1}{k_{31}}[\exp[-k_{31}(T_b-T_L)]-1]\right\} \qquad \text{(Eq. 12)}$$

and:

$$\lambda = E_1 = k_{12} + k_{10} \qquad \text{(Eq. 13)}$$

RESULTS AND DISCUSSION

In the real case with real eigenvalues, the response function for Model 1 consists of three regular exponential terms. However, in the complex case (Eq. 10), the function consists of two terms: an exponentially damped, oscillating trigonometric term superimposed on a regular exponential term. Thus, for a linear system behaving according to Model 1 and showing significant reabsorption, one may believe that the response function will show secondary peaks or humps judged from the functional form of Eq. 10. However, this does not appear to be the case. Although the curve fittings for all subjects using Eq. 2 lead to a convergence with complex eigenvalues (Eq. 10), no secondary peaks or humps were observed in the fitted curves (Fig. 1).

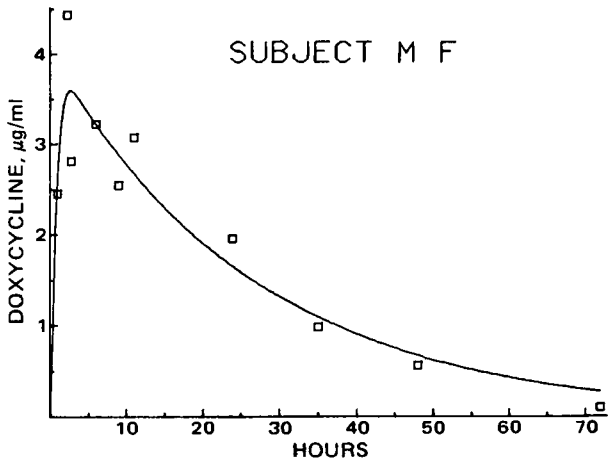


Figure 1—*Least-squares fit of Model 1 (Eq. 2) to plasma doxycycline level data. The fitting resulted in complex eigenvalues, with an estimated response function as given by Eq. 10.*

Extensive simulation studies were done on an interactive computer graphic system to investigate whether any perturbation of the microparameters of the model may result in secondary peaks or humps in the regression curve. Apparently, this effect does not occur even for systems with a substantial degree of reabsorption or for systems constructed numerically by maximizing the "degree of complexity" on the basis of the discriminant of the characteristic polynomial.

From a purely theoretical point of view, some support apparently exists for the claim that linear systems with cyclic pathways and continuous transfer do not give rise to secondary peaks and that humps may be difficult to detect, but there is no formal proof (13). This behavior is related to a relatively long period of the trigonometric expression and a relatively strong exponential damping term inherent in such systems.

When dealing with cyclic models with complex eigenvalues, as opposed to noncyclic systems, it is improper to fit the macroparametric form instead of the microparametric form of the response function. For example, if Eq. 10 is fitted in its macroparametric form, the curve may approximate an extra peak or a hump in the data. However, if the macroparameters are used to calculate the microparameters, then some of them may turn out to be complex or negative, in disagreement with the model proposed.

Table I shows that the reabsorption rate constant k_{23} is relatively large compared to the elimination rate constant k_{el} and k_{12} for all subjects, indicating substantial reabsorption. In spite of this significant reabsorption, the fitted curves do not appear distinguishable from ordinary absorption curves for noncyclic linear systems (Fig. 1). Reabsorption and cyclic transport processes in pharmacokinetics have usually been considered only when dealing with data containing secondary peaks or an extra hump. These results show that a "typical" drug level profile may be associated with a strong degree of reabsorption. Therefore, a greater number of drugs could be subject to reabsorption than currently known.

The average absorption half-life estimated using Model 1 is 0.5 hr (Table I), which is considerably shorter than the 5.3 hr determined by the method of residuals by Gibaldi (1) but comparable to the half-life of 50 min determined by Schach von Witteman (2). The average elimination half-life of 13 hr is larger than the 9–8 hr determined by Gibaldi (1) but considerably shorter than that determined by Schach von Witteman (2). These differences are mainly due to the different models used. Gibaldi (1) suggested that the slow decline of plasma doxycycline levels relative to other tetracyclines is due to relatively slow absorption compounded by enterohepatic cycling. However, the absorption and elimination half-lives were calculated assuming a one-compartment model without considering reabsorption (1, 2).

[1] The model contains a cyclic pathway. Thus, neither the Benet approach (7) nor the Vaughan *et al.* approach (8, 9) can be used for this reabsorption model.

Table I—Least-Squares Parameter Estimates for Model 1

Parameter	Subject						Mean	CV, %
	JN	CS	RV	MF	FF	GP		
k_a[a], hr^{-1}	3.27	1.82	1.52	1.03	0.678	2.79	1.85	54.4
$t_{1/2}$(abs), hr^{-1}	0.212	0.381	0.456	0.673	1.02	0.248	0.499	61.2
k_{el}[b], hr^{-1}	0.0446	0.0632	0.0473	0.0548	0.0770	0.0502	0.0562	21.6
$t_{1/2}$ el, hr	15.5	11.0	14.7	12.6	9.00	13.8	12.8	19.1
T_L, hr	2.15	0.719	0.108	0.131	1.13	0.122	0.727	111
k_{12}, hr^{-1}	0.546	0.590	0.355	0.245	0.217	0.392	0.391	39.1
k_{23}, hr^{-1}	1.13	2.09	1.31	1.19	0.643	1.76	1.354	37.5
D_F/V, g/ml	4.20	4.62	4.71	5.77	4.79	4.13	4.70	12.5
RSS[c]	0.393	0.322	1.22	1.83	0.298	0.242	0.718	91.5
AIC[d]	2.67	0.679	14.0	18.0	−0.091	−2.21		

[a] $k_a = k_{31}$. [b] $k_{el} = k_{12} + k_{10}$. [c] RSS = residual sum of squares. [d] Akaike's information criterion.

Table II—Least-Squares Parameter Estimates for Model 2

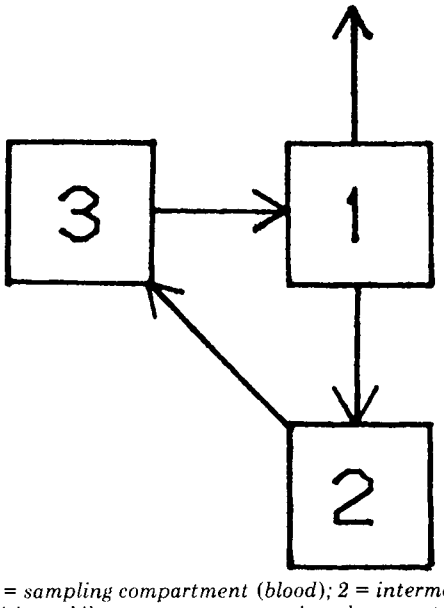
Parameter	Subject						Mean	CV, %
	JN	CS	RV	MF	FF	GP		
k_a[a], hr^{-1}	1.04	6.34	5.29	3.67	0.619	2.89	3.31	68.7
$t_{1/2}$(abs), hr	0.666	0.109	0.131	0.189	1.12	0.240	0.409	98.7
k_{el}[b], hr^{-1}	0.499	0.553	0.0407	0.050	0.067	0.044	0.0511	18.2
$t_{1/2}$ el, hr	13.9	12.5	17.0	13.9	10.3	15.8	13.9	16.9
T_L, hr	1.99	0.904	0.711	0.737	0.915	0.024	0.880	72.1
T_b, hr	23.8	16.4	9.27	10.8	24.7	5.91	15.2	51.7
F_b	0.570	0.488	0.436	0.952	0.368	0.338	0.525	42.8
k_{12}, hr^{-1}	0.0568	0.0624	0.0777	0.0412	0.0743	0.103	0.0692	30.5
D_F/V, g/ml	2.94	3.00	3.07	3.97	3.12	2.92	3.17	12.6
RSS[c]	0.0898	0.139	0.866	1.24	0.136	0.0875	0.426	117.3
AIC[d]	−10.1	−5.71	12.6	16.2	−6.0	−10.4		

[a] $k_a = k_{31}$. [b] $k_{el} = k_{12} + k_{10}$. [c] RSS = residual sum of squares. [d] Akaike's information criterion.

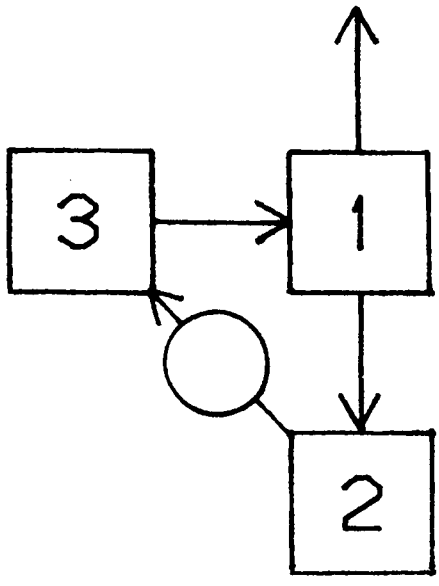
It appears that plasma level data showing secondary peaks or extra humps are not well approximated by linear compartment models with continuous first-order intercompartment drug transfer. However, such behavior may be related to discontinuous transfer processes. The doxycycline data appear to show secondary peaks that are not described by Model 1. Model 2 is identical to Model 1 except that drug transfer back into the intestine is a discontinuous bolus transfer *via* the bile. This model appears physiologically more meaningful than Model 1, considering the discontinuous nature of biliary secretion (5).

The average absorption half-life of 0.4 hr is somewhat smaller than that obtained using Model 1 (0.5 hr) while the average elimination half-life of 14 hr is greater than the 13 hr previously determined (Tables I and II). Although the average absorption and elimination half-lives are not very different from those determined using Model 1, Model 2 fits the data consistently better than Model 1 judged from the residual sum of squares and the Akaike information criterion values (Table II and Fig. 2).

Almost complete absorption takes place from the upper intestine following oral doxycycline administration (4). To account for the 70–80% doxycycline eventually recovered in the feces (14), intestinal secretion has been proposed as primarily responsible with biliary secretion playing a minor role (14, 15). It was proposed that after essentially complete absorption, the drug in the blood diffuses into the alkaline medium of the intestinal lumen where cationic chelation occurs, in which form doxycycline cannot be reabsorbed and is ultimately eliminated in the feces (16). Some investigations indicated that average concentrations of doxycycline are about five times greater than serum concentrations and that bile concentrations of the gallbladder are 10–15 times greater than serum concentrations (4). It was estimated that 10–15% of a 200-mg dose



Model 1—1 = sampling compartment (blood); 2 = intermediate compartment, either a bile compartment or a virtual compartment representing a secreted nonabsorbable compound or complex from which an absorbable form of the drug is released; and 3 = compartment from which absorption takes place.



Model 2—1 = sampling compartment (blood); 2 = bile compartment from which bile containing drug is released spontaneously as a bolus squirt to the absorption compartment; and 3 = compartment from which absorption takes place.

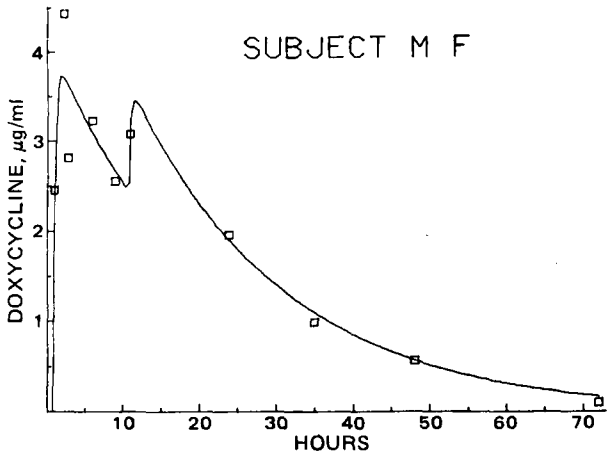


Figure 2—*Least-squares fit of Model 2 (Eq. 11) to plasma doxycycline level data.*

would be eliminated in the bile per day if an average of 500 ml of bile was secreted (4). If doxycycline's metabolism rate, urinary excretion rate, and excretion rate of chelate complex are first-order processes, or if the combined elimination rate by these processes is approximately first order (k_{10}), then Model 2 may be physiologically meaningful if the absorption rate is first order.

In humans, bile is retained in the gallbladder until it is spontaneously released, usually after a meal (5). The estimated times of bile release, T_b, in six subjects varied from 5 to 24 hr. Food and especially fats promote contraction of the gallbladder so that the timing and fat content of the meal play a role in the estimated time and magnitude of bile release.

It is difficult to speculate about the feasibility of these times and the degree of reabsorption because of the limited data and the inaccuracy of the assay methodology and because no specific information is available about the food intake of the subjects after drug administration. However, the data appear to show a consistent trend of reabsorption, and the Akaike information criterion (17, 18) seems to indicate, in comparing the two linear compartment models (Tables I and II), that the reabsorption mechanism is discontinuous rather than continuous.

NOTATIONS

c = drug concentration in sampleable compartment (blood plasma) at time t

D_F = amount of dose available in the compartment where absorption takes place

$E_i = \sum_{j=0}^{n} k_{ij}$, where $n = 3$

$\bar{f}_i$ = Laplace transform of input rate into the ith compartment

F_b = fraction of the amount of drug cumulated in the bile that is released by a squirt of bile at time $t = T_b$

$Im(z)$ = imaginary part of complex number z

k_{ij} = first-order rate constant for drug transfer from compartment i to compartment j

L^{-1} = inverse Laplace transform operator

λ = eigenvalue of linear pharmacokinetic system (real or complex)

$Re(z)$ = real part of complex number z

s = Laplace transform variable

$|\mathbf{S}|$ = $|\mathbf{S}(s)|$, determinant of $\mathbf{S}$ matrix defined in Ref. 6.

$|\mathbf{S}|_{ij}$ = $|\mathbf{S}(s)|_{ij}$ cofactor corresponding to the i,jth position of $\mathbf{S}$ matrix

T_b = time when bile containing drug is released to absorption compartment

T_L = absorption lag time

V = volume of distribution of sampleable compartment

x_i = amount in ith compartment at time t

$(y)_+$ = max(0,y), the "truncation function"

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