half-life, as is generally accepted as optimum therapy from a pharmacokinetic point of view, deviations occur even when k_a/β is large. When the dosing interval is decreased the deviations become greater. When k_a/β approached unity, as would be expected from certain controlled-release dosage forms, the deviations become enormous. In addition, it must be realized that although R_2 more closely reflects R_1 values than does R_3, it is not a predictive method, in that one must achieve steady state to determine $C_{min(ss)}$, whereas R_1 and R_3 can be used predictively following a single dose. These variables must be kept in mind when one is attempting to anticipate or predict drug accumulation and consequent pharmacological effects from single-dose data.

(1) J. H. Hull, *J. Clin. Pharmacol.*, **20**, 644 (1980).
(2) M. Gibaldi and D. Perrier, in "Pharmacokinetics," Dekker, New York, N.Y. 1975, p. 115.
(3) D. J. Greenblatt, M. Divoll, J. S. Harmatz, D. S. MacLaughlin, and R. I. Shader, *Clin. Pharmacol. Ther.*, **30**, 475 (1981).
(4) P. J. Perry, B. Alexander, F. J. Dunner, R. D. Schoenwald, B. Pfohl, and D. Miller, *J. Clin. Psychopharmacol.*, **2**, 114 (1982).

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Fraction Unbound in Interstitial Fluid

Keyphrases □ Pharmacokinetics—fraction unbound in interstitial fluid, relationship between binding in interstitial and vascular space

To the Editor:

The influence of protein binding of drugs on their pharmacological effect and pharmacokinetic disposition has been widely studied (1–3). However, most of the experimental observations have involved the interaction of drugs with plasma or serum proteins and not extravascular proteins. The lack of useful, experimental observations in this area is a result of the difficulty in obtaining representative tissue samples and the inadequate methods for performing tissue-binding studies (4). A mathematical approach has been derived (5) for estimating the fraction unbound in the "tissue" space utilizing a calculated volume of distribution term and anticipating physiological spaces. However, this approach is limited and only provides a complex average fraction unbound located outside the vascular space. Therefore, alternative theoretical approaches, as well as experimental methods need to be developed.

Nowhere is the role of plasma and tissue binding of greater interest than in the area of antibiotic therapy (6, 7). In general, β-lactam antibiotics are restricted in their distribution to the vascular space and the interstitial fluids; they do not penetrate intracellularly. Attempts to study the tissue (interstitial) binding of these antibiotics have centered on the collection of fluid from tissue cages (8), but the physiological character of the collected fluid has been questioned (9). The purpose of the present communication is to derive a theoretical relationship that relates the binding of a drug in the extravascular–extracellular or interstitial space to the binding in the vascular space. The original model on which this work is based was put forth by Øie *et al.* (10) and was recently used to describe the distribution of ceftriaxone (11). This theoretical relationship is used to explain the lack of distributional changes occurring with ceftriaxone despite the dramatic changes in the fraction unbound in the plasma (11).

The interaction of drugs with plasma proteins is usually described by the following Langmuir binding isotherm:

$$C_{BP} = \sum_{i=1}^{m} \frac{n_i P^* C_U}{Kd_i + C_U} \qquad \text{(Eq. 1)}$$

where C_{BP} is the plasma concentration of bound drug, m is the number of classes in binding sites, n_i is the number of binding sites for the ith class of binding sites, P is the concentration of the binding protein located in the vascular space, C_U is the concentration of unbound drug, and Kd_i is the equilibrium dissociation constant for the ith class of binding sites.

The presence of plasma proteins (*i.e.*, albumin) in the interstitial fluids has been well documented (12). If one assumes that the drug–protein interaction in the interstitial space is identical to the interaction in the vascular space (equivalent capacity and affinity constants), then a similar Langmuir relationship can be written for the interstitial binding:

$$C_{BE} = \sum_{i=1}^{m} \frac{n_i E^* C_U}{Kd_i + C_U} \qquad \text{(Eq. 2)}$$

where C_{BE} is the interstitial concentration of the bound drug and E is the concentration of binding protein in the interstitial space.

Equation 1 can be rewritten to factor out the protein and unbound concentration to yield:

$$C_{BP} = P^* C_U^* \sum_{i=1}^{m} \frac{n_i}{Kd_i + C_U} \qquad \text{(Eq. 3)}$$

For ease of manipulation, let a new parameter, S, replace the summation term:

$$C_{BP} = P^* C_U^* S \qquad \text{(Eq. 4)}$$

Given the assumptions concerning equivalent binding proteins in the vascular and interstitial spaces, and the additional assumptions of: (*a*) equal unbound drug concentration in both physiological spaces; (*b*) Kd_i does not change at lower protein concentrations; (*c*) other mechanisms of tissue distribution such as active transport, selective membrane permeability, and ion trapping are not present, then the S term for both C_{BP} and C_{BE} are equal, and a similar rearrangement and substitution can be written for C_{BE}:

$$C_{BE} = E^* C_U^* S \qquad \text{(Eq. 5)}$$

By definition, the fraction unbound in the plasma or vascular space (f_P) may be written as:

$$f_P = \frac{C_U}{C_U + C_{BP}} = \frac{1}{1 + P^* S} \qquad \text{(Eq. 6)}$$

A similar fraction unbound in the interstitial space (f_E) may be written as:

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$$f_E = \frac{1}{1 + E^*S} \quad \text{(Eq. 7)}$$

One can rearrange Eq. 6 to obtain the S parameter in terms of f_P and P via:

$$S = \frac{1 - f_P}{P^* f_P} \quad \text{(Eq. 8)}$$

Substituting Eq. 8 into Eq. 7, one can now write an expression for f_E in terms of f_P, P, and E:

$$f_E = \frac{1}{1 + \frac{E}{P}\left(\frac{1 - f_P}{f_P}\right)} \quad \text{(Eq. 9)}$$

Figure 1 illustrates the theoretical relationship between f_P and f_E, when the ratio of protein concentration (E/P) is normal (0.32), less than normal (0.10), or greater than normal (0.64) (12). Two distinct trends are apparent from the data. As might be expected, decreasing or increasing the relative concentration of interstitial protein results in a larger or smaller fraction unbound in the interstitial space (f_E) relative to the vascular space (f_P). A second trend is the observation that as f_P values <0.1 the relationship between f_E and f_P becomes linear, irrespective of the value of E/P. This fixed ratio of f_E to f_P can be explained readily if one evaluates Eq. 9 as f_P approaches zero.

In this case:

$$f_E = \frac{P}{E} {}^* f_P \quad \text{(Eq. 10)}$$

Therefore, for drugs that are highly bound, the ratio of their fraction unbound in the interstitial and vascular spaces (f_E/f_P) is inversely related to the ratio of the concentration of binding protein located in those physiological spaces (E/P). Furthermore, any increases in f_P will result in proportional changes in f_E, such that the ratio of the two drugs (f_E/f_P) will remain relatively constant at f_P values <0.1.

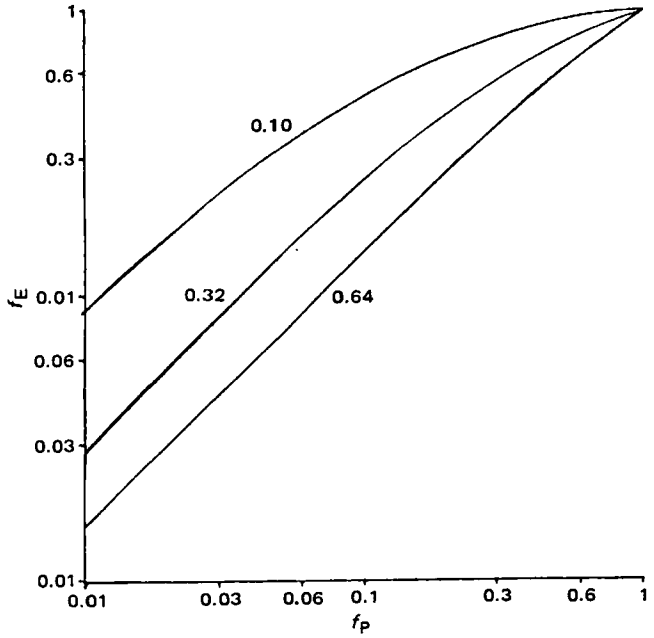

Figure 1—*Interrelationship of f_E and f_P as defined by Eq. 9; inset numbers refer to* E/P *values.*

One of the important implications for this theoretical observation lies in the distribution of drug mass. The pharmacokinetic parameter which best reflects shifts in drug distribution is the apparent volume of distribution at steady state calculated with reference to total drug levels (V_{ss}^T). As mentioned above, we have previously put forth a conceptual model or approach based on the work of Gillette (13) which relates V_{ss}^T to drug binding and physiological spaces (5). The following equation represents a modification of the original work:

$$V_{ss}^T = V_P + \frac{f_P}{f_E} V_E \quad \text{(Eq. 11)}$$

where V_P and V_E are the physiological volumes of the plasma and interstitial spaces, respectively.

Table I illustrates the influence of the ratio of the fractions unbound (f_E/f_P) on V_{ss}^T as f_P increases. In agreement with the observed ceftriaxone data (where f_P increased from 0.03 to 0.1), the change in V_{ss}^T is very small. Therefore, for this particular system under the stated conditions, one should expect no substantial shift in drug mass out of the vascular compartment as a result of an increase in f_P.

The stable nature of V_{ss}^T is contrasted by the large changes that occur in the parameter V_{ss}^U (Table I). The V_{ss}^U parameter can be derived experimentally from the analysis of the unbound concentration *versus* time profile (11) and has been theoretically described by the following equation:

$$V_{ss}^U = \frac{V_P}{f_P} + \frac{V_E}{f_E} \quad \text{(Eq. 12)}$$

As f_P and f_E increase, V_{ss}^U decreases strikingly. These dramatic changes in V_{ss}^U **do not** reflect any changes in the distributional space. They merely reflect the substantial shift in the ratio of unbound concentration in the plasma to the amount of drug in the body (*i.e.*, dose). The utility of V_{ss}^U is described elsewhere (11).

In conclusion, we have presented a conceptually useful equation which describes the fraction unbound in the interstitial space and provides a theoretical explanation for the observed behavior of the apparent volume of distribution for ceftriaxone. The equation was developed for

Table I—Influence of Increasing f_P Values and Various Ratios of E/P on V_{ss}^T and V_{ss}^U as Calculated by Eqs. 9, 11, and 12 [a]

		V_{ss}^T ml/kg		
f_P	E/P	0.10	0.32	0.64
0.001		59.2	96.5	147.
0.005		59.8	97.0	148.
0.01		60.5	97.6	148.
0.05		66.7	102.	151.
0.1		74.3	108.	154.
0.5		136.	154.	180.
1.0		212.	212.	212.
		V_{ss}^U liter/kg		
f_P	E/P	0.10	0.32	0.64
0.001		59.2	96.6	147.
0.005		12.0	19.4	30.2
0.01		6.05	9.76	15.1
0.05		1.33	2.04	3.08
0.1		0.75	1.08	1.57
0.5		0.27	0.31	0.36
1.0		0.21	0.21	0.21

[a] V_P = 42 ml/kg; V_E = 170 ml/kg.

antibiotics, but may have wider application. However, this application must be preceded by an appreciation (and if possible the testing) of the underlying assumptions of this relationship. The major assumption remains that the only binding that occurs outside the vascular space is to plasma proteins located there, and that the binding constants remain the same.

(1) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).
(2) A. Yacobi and G. Levy, *J. Pharm. Sci.*, **64**, 1660 (1975).
(3) G. H. Evans, A. S. Nies, and D. G. Shand, *J. Pharmacol. Exp. Ther.*, **180**, 114 (1973).
(4) W. J. Jusko and M. Gretch, *Drug Metab. Rev.*, **5**, 43 (1976).
(5) M. Gibaldi and P. J. McNamara, *J. Pharm. Sci.*, **66**, 1211 (1977).
(6) W. A. Craig and C. M. Kunin, *Annu. Rev. Med.*, **27**, 287 (1976).
(7) W. A. Craig and P. G. Welling, *Clin. Pharmacokinet.*, **2**, 252 (1977).
(8) G. D. Chisholm, *Scand. J. Infect. Dis. Suppl.*, **14**, 118 (1978).
(9) H.-U. Eickenberg, *Scand. J. Infect. Dis. Suppl.*, **14**, 166 (1978).
(10) S. Øie, T. W. Guentert, and T. N. Tozer, *J. Pharm. Pharmacol.*, **32**, 471 (1980).
(11) P. J. McNamara, M. Gibaldi, and K. Stoeckel, *Eur. J. Clin. Pharmacol.*, submitted.
(12) T. Kawai, in "Clinical Aspects of the Plasma Proteins," Lippincott, Philadelphia, Pa., 1973, pp. 114–130.
(13) J. R. Gillette, *Ann. N.Y. Acad. Sci.*, **179**, 43 (1971).

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Creatinine XII: Comparison of Assays of Low Serum Creatinine Levels Using High-Performance Liquid Chromatography and Two Picrate Methods

Keyphrases □ Creatinine—assay in serum using HPLC, automated picrate method, modified picrate method, comparison of methods □ High-performance liquid chromatography—creatinine assay in serum, comparison with two picrate methods

To the Editor:

Endogenous creatinine has been commonly employed to estimate the glomerular filtration rate for the study of renal function or for modifying dosages in patients with renal impairment (1–5). Clinically, the automated picrate method[1], based on a complex color reaction between creatinine and picrate in the alkaline medium, is probably most widely used to assay creatinine in plasma or serum (6–10). This method, however, is known to be nonspecific due to potential interferences by endogenous and/or exogenous substances, and often results in an overestimate of "true" creatinine levels (7–9). It appears that to date most of assay comparisons between this method and the more specific high-performance liquid chromatographic (HPLC) method were carried out in samples containing higher levels (such as >0.8 mg%) of creatinine (10, 11). Since lower levels are often found in patients, it would seem important to evaluate potential discrepancies using low-level samples. The modified picrate method of Yatzidis (12, 13) was also chosen for the present evaluation, since it has been reported to be highly specific.

A total of 30 random serum samples from patients determined by the automated picrate method in a clinical laboratory[2] and found to contain less than 0.8 mg% of creatinine, were employed in the study. The HLPC method (14) used in the present study is a slight modification of the method developed earlier in our laboratory (3). Briefly, the method involved the deproteinization of 0.1 ml of serum with 0.25 ml of acetonitrile. After vortexing and centrifugation, 50 μl of the supernatant was injected directly into the cation-exchange column[3]. The recovery in the above sample preparation is essentially 100% (3). The mobile phase with a flow rate of 3 ml/min contained 0.035 *M* monobasic ammonium phosphate adjusted to pH 4.8 with 0.01 *N* NaOH. The creatinine was monitored at 254 nm using a fixed wavelength detector[4] with a sensitivity setting of 0.005 AUFS. The retention time for creatinine was about 4.5 min. The present assay has a detection limit of 0.05 mg% (based on a signal/noise ratio of 3.0), and has a coefficient of variation for both interassay and intraassay between 1.2 and 3.0%. No interferences were found in the present and earlier (14) studies with samples obtained from patients or volunteers. It should be noted that the variable wavelength UV detector (without a noise damper) used in our earlier studies (3, 10) was much less sensitive; it had a larger base-line noise even at a previously used sensitivity setting of 0.05 AUFS. Duplicate analyses were performed using both the HPLC method and the Yatzidis method.

The results of the serum creatinine measured by the above three methods are summarized in Table I. The automated method overestimated serum creatinine by an average of 15.2% with a −16.7 to 66.7% range when compared with the HPLC method. Although the mean overestimation found in the present study is similar to the previous study (14.5%, $n = 30$) using samples with generally much higher serum levels (10), it is of interest to note (Table I) that for two samples the overestimations were >50%, and for eight samples the results were essentially identical. The above results suggest that the amount and nature of interfering substances may vary considerably with individuals.

The overestimations of serum creatinine by the modified picrate method were much higher with an average of 55.2% (ranging from −58.3 to 168%). In the previous report (10), a similar modified method (15) was found to overestimate

[1] Auto Analyzer SMA 6/60, Technicon Instruments, Tarrytown, N.Y.

[2] University of Illinois Hospital, Chicago, Ill.
[3] Partisil PXS 10/25 SCX, 30 cm, Whatman Inc., Clifton, N.J.
[4] Model 440, Waters Associates, Milford, Mass.

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