J. vet. Pharmacol. Therap. **27**, 441–453, 2004. REVIEW

Volumes of distribution

P. L. TOUTAIN &
A. BOUSQUET-MÉLOU

UMR 181 Physiopathologie et Toxicologie Expérimentales INRA/ENVT, Ecole Nationale Vétérinaire de Toulouse, Toulouse cedex 03, France

Toutain, P. L., Bousquet-Mélou, A. Volumes of distribution. *J. vet. Pharmacol. Therap.* **27**, 441–453.

Volumes of distribution are proportionality constants between total amount of drug in the body and plasma concentrations. As snapshot plasma drug concentrations may be measured in different conditions (at equilibrium, under pseudo-equilibrium condition,…), several volumes of distribution have been defined. The two most relevant are the volume of distribution at equilibrium (V_{ss}), and the volume of distribution during pseudo-equilibrium (V_{area}). Volumes of distribution are used to compute a loading dose (V_{ss}) or the residual amount of drug in the body knowing plasma concentrations (V_{area}). Volume of distribution may be interpreted in terms of drug distribution having recourse to physiological models involving drug binding to plasma and tissues. Volumes of distribution should be determined early in drug development programmes and those having a large volume of distribution may be selected to obtain a long terminal half-life even for drugs having a relatively high clearance.

P. L. Toutain, UMR 181 Physiopathologie et Toxicologie Expérimentales INRA/ENVT, Ecole Nationale Vétérinaire de Toulouse, 23, chemin des Capelles, 31076 Toulouse cedex 03, France. E-mail: pl.toutain@envt.fr

INTRODUCTION

Three volumes of distribution (V_d) are classically reported in the scientific literature: the volume of the central compartment (V_c), the volume of distribution calculated by the area method (V_{area}) and the steady-state volume of distribution (V_{ss}).

V_d is the parameter used to assess the amount of drug in the body from the measurement of a snapshot plasma concentration. The main clinical application of V_d is to compute a loading dose (e.g. the first dose of a multiple dosage regimen) in order to immediately reach the target therapeutic plasma concentration. Frequently, and often incorrectly, the numerical value of a V_d is advocated to support claims on the extent of drug distribution. It should be stressed that V_d was not primarily designed to evaluate drug distribution in the different physiological spaces, and that a V_d can be much higher than the total body water space (Fig. 1). Nevertheless, a physiological interpretation of V_d is possible but this requires having recourse to models involving drug binding to plasma and tissues.

This review defines each volume of distribution, how to compute them (with particular attention to the most frequent incorrect computations) and how to interpret and use appropriately the different V_d.

VOLUME OF DISTRIBUTION: A GENERAL DEFINITION

All the volumes of distribution correspond to the ratio of an amount (A) of drug in the body at a given time (A_t), and plasma (blood) concentration at that time (Eqn 1):

$$V_d = \frac{\text{Amount of drug in the body at time } t\ (A_t)}{C_{plasma} \text{ at time } t} \qquad (1)$$

By definition a V_d should only be regarded as a proportionality constant (parameter) between a plasma concentration and the corresponding amount of drug in the body. This proportionality constant having a volume for dimension has been termed volume of distribution. Figure 2 gives a pictorial view of the concept of V_d using a flask analogy to the body.

DRUG DISTRIBUTION AND VOLUME OF DISTRIBUTION

The evaluation of drug distribution (with its different determinants) and that of volume of distribution are two different issues. They should be carefully distinguished, even if relationships between them exist, as expressed by different physiological models (*vide infra*). Figure 3 depicts the principles of drug

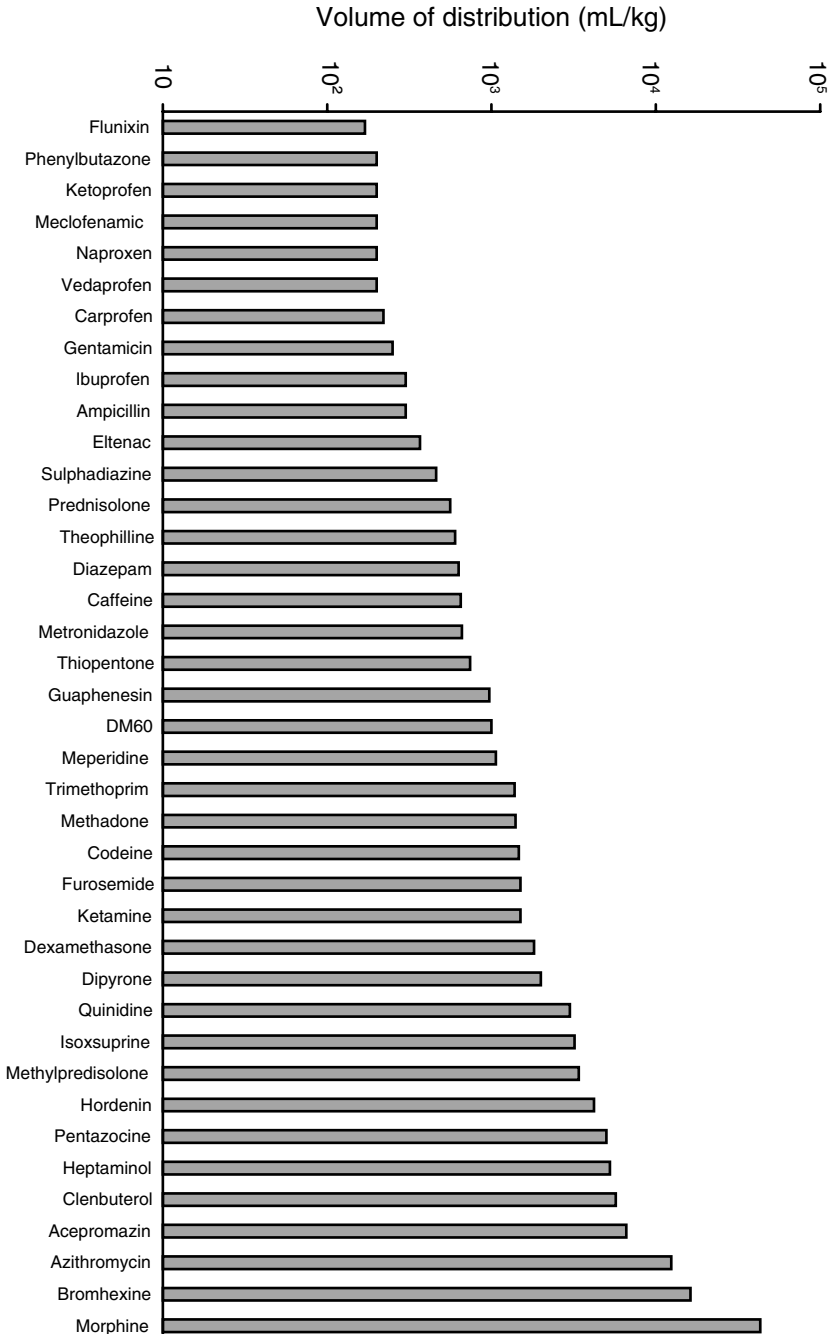


Fig. 1. Volume of distribution, V_{area} (mL/kg), for a selection of drugs in the horse.

repartition between the vascular space (plasma) and extravascular spaces (tissues).

WHY DO SEVERAL VOLUMES OF DISTRIBUTION EXIST?

As the plasma concentration can be measured in different situations [just after an intravenous (i.v.) drug administration, during the phase of drug distribution, during the terminal phase of drug disposition or at equilibrium], several V_d are needed because the proportionality ratio between the amount of drug in the body and the plasma concentration will have different values according to the state of drug disposition. Figure 4 gives the four possibilities, with V_c being the initial volume of distribution, V_{ss}, the appropriate volume of distribution when plasma concentrations are measured in steady-state conditions, and V_{area} or V_z (formerly termed $V_d\beta$), the appropriate V_d when plasma concentration is measured in pseudo-equilibrium conditions. When plasma concentration is measured during the drug distribution phase, the ratio of the drug amount over the plasma concentration is not a parameter but a time dependent variable.

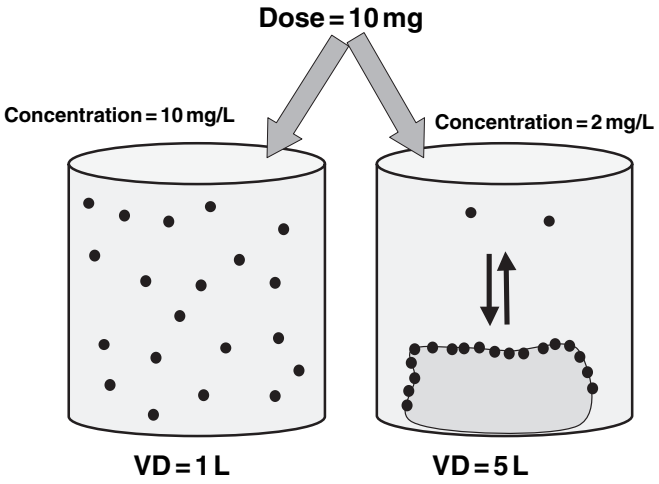


Fig. 2. A simplistic system to introduce the concept of volume of distribution. The same amount of drug (10 mg) is dissolved in the same volume of water (1 L). For the left beaker the water concentration is 10 μg/mL and when applying Eqn 1 in the text, the computed apparent volume of distribution of the drug in the left beaker is 1 L (which corresponds to the actual volume of water in the beaker). In the right beaker, there is charcoal that can fix part of the drug, which is divided between water and charcoal. The water concentration is here of only 2 μg/mL and applying Eqn 1 of the text gives an apparent volume of distribution of 5 L, i.e. five times the actual volume of water.

THE INITIAL (V_C) AND THE TERMINAL (V_{AREA} OR V_Z) VOLUMES OF DISTRIBUTION AFTER INTRAVENOUS BOLUS DRUG ADMINISTRATION

Just after an i.v. drug administration, plasma concentration is maximal (C_0). Before any drug elimination or distribution, the amount of drug in the body is by definition equal to the administered dose, and the plasma concentration is C_0. Applying the definition of a V_d (Eqn 1), the initial volume of distribution (V_c) is (Eqn 2):

$$V_c = \frac{\text{Dose}}{C_0} \tag{2}$$

Equation 2 assumes that C_0 corresponds to an initial plasma concentration resulting from a total drug mixing in blood before any drug elimination or distribution, which is generally an unrealistic assumption. Practically speaking, C_0 is estimated by extrapolation to time zero of the drug disposition curve (Fig. 5 gives an example of the determination of C_0 for the evaluation of plasma volume using Evans blue).

In the framework of a compartmental analysis, the initial volume of distribution is termed volume of the central compartment and is obtained by mean of Eqn 3:

$$V_c = \frac{\text{Dose}}{\sum_{i=1}^{n} Y_i} \tag{3}$$

where Y_i are intercepts of the different phases of the kinetic disposition obtained by fitting the plasma drug concentration vs. time profile.

Therefore, V_c can be viewed as the apparent volume from which drug elimination occurs because kidney and liver, the two main clearing organs, belong to the central compartment (Fig. 6).

Immediately after i.v. bolus administration, the drug begins to be distributed and eliminated. If the distribution is instantaneous, after the drug administration, the body is reducible to a homogenous pool (compartment) and V_c remains the single V_d. However, for the vast majority of drugs, there is a non-instantaneous distribution phase during which the drug plasma concentration decreases more rapidly than the total amount of drug actually present in the body. During the distribution phase, the decrease of plasma concentration is largely due to the partitioning of the drug in the body, and not to the drug elimination. Therefore, according to the definition of a V_d (Eqn 1), the latter increases progressively until it reaches an asymptotic value when the pseudo-equilibrium of distribution is achieved. This asymptotic value is termed V_{area}. Thus, V_d during the distribution phase can be viewed as a time-dependent variable that expands from V_c immediately after the bolus drug administration until it reaches the maximal value of V_{area} (Fig. 7).

When the distribution pseudo-equilibrium is reached, the net exchange (balance) between plasma (central compartment) and the tissues (peripheral compartments) is null, and the decrease of plasma concentration is now only because of irreversible drug elimination, which is proportional to the body (total) clearance (Eqn 4):

$$\text{Rate of drug elimination} = Cl_{tot} \times C_{plasma} \tag{4}$$

where Cl_{tot} is the plasma clearance.

The relationship between plasma concentration and the amount of drug in the body during the elimination phase is given by Eqn 5:

$$V_{area} = \frac{\text{Amount of drug in the body during the terminal phase}}{\text{Plasma concentration during the terminal phase}} \tag{5}$$

The amount of drug present in the body at a given time t during the elimination phase is equal to the amount of drug which remains to be eliminated, i.e. (Eqn 6):

$$\text{Amount of drug in the body at time } t_i = \int_{t_i}^{\infty} Cl \times C_{(t)}\,dt \tag{6}$$

where t_i is a time during the elimination phase. Integration of this equation gives Eqn 7:

$$\text{Amount of drug at time } t_i = Cl \times [AUC_{(t_i-\infty)}] \tag{7}$$

where $AUC_{(t_i-\infty)}$ is the area under the plasma concentration vs. time curve between t_i and infinity.

During the terminal phase the plasma concentration decays according to a mono-exponential curve which can be described by a mono-exponential equation (Eqn 8) :

$$C(t_i) = Y_z \exp(-\lambda_z t_i) \tag{8}$$

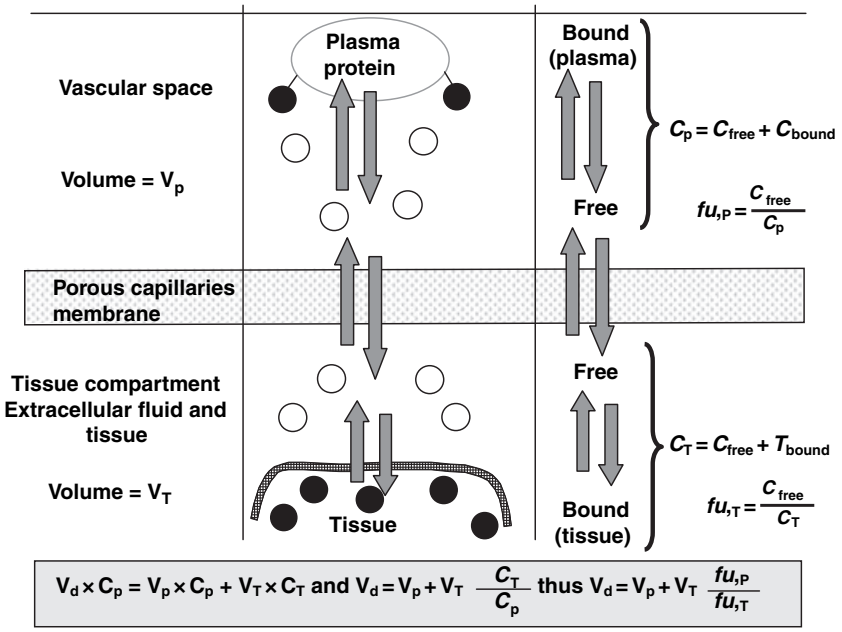


Fig. 3. Principles of drug partition between vascular space (plasma) and non-vascular space (tissue), and establishment of a minimal model for volume of distribution. Upper panel: in plasma (volume: V_P), drug is either free (open circle) or bound (black circle) to proteins or red blood cells. The total concentration in plasma (C_P) is the sum of bound (C_{bound}) and free (C_{free}) concentrations. The free fraction in plasma ($fu,_P$) is the ratio C_{free}/C_P. Lower panel: only the free drug (open circle) can cross the capillary membrane and gain access to the extracellular fluids outside the plasma. In extra-vascular space (volume V_T), drug is also either free (open circle) or bound (black circle) to extra-vascular components; when equilibrium is achieved, the unbound concentration (C_{free}) in the vascular and extra-vascular spaces are the same and the free fraction in the extra-vascular space ($fu,_T$) is the ratio C_{free}/C_T; C_T being the total concentration in extracellular space, i.e. C_{free} + T_{bound}. According to the principle of mass balance, the total amount of drug in the body is equal to the amount in plasma (V_PC_P) plus the amount in tissue (V_TC_T). By definition of volume of distribution (see Eqn 1):

$$V_d = \frac{V_P C_P + V_T C_T}{C_P} \text{ or } V_d = V_P + V_T \times \frac{C_T}{C_P}$$

Considering that C_T/C_P is equal to $fu,_P / fu,_T$:

$$V_d = V_P + V_T \times \frac{fu,_P}{fu,_T}$$

Total concentrations in plasma and tissue spaces are functions of the affinities of the drug in these spaces for the different components to which drug binds, and it is the free drug concentration which 'controls' the total drug concentration, not the reverse.

where $C(t_i)$ is the predicted plasma concentration at time t_i, Y_z and λ_z are the intercept and the slope of the terminal phase, respectively.

$AUC_{(t_i-\infty)}$ can be easily computed by integrating Eqn 8 from t_i to infinity (Eqn 9):

$$AUC_{(t_i-\infty)} = \frac{C(t_i)}{\lambda_z} \quad (9)$$

Now applying the definition of V_{area} (Eqn 5) and combining Eqns 7 and 9 gives (Eqn 10):

$$V_{area} = \frac{Cl \times [C(t_i)/\lambda_z]}{C(t_i)} \quad (10)$$

and after simplification (Eqn 11) :

$$V_{area} = \frac{Cl}{\lambda_z} = \frac{\text{Dose}}{AUC_{(0-\infty)}} \times \frac{1}{\lambda_z} \quad (11)$$

Inspection of Eqn 11 explains why this V_d is termed V_{area} or V_z. More importantly, Eqn 11 shows that V_{area} is a volume term that is tributary of plasma clearance, and explaining that the numerical value of V_{area} can be modified by an alteration of plasma clearance, whereas the actual physiological space of the drug repartition remains unchanged (*vide infra*).

THE ERRONEOUS COMPUTATIONS OF V_{AREA} FOR EXTRA-VASCULAR ROUTES

The computation of V_{area} requires two major assumptions: (i) the dose which gains access to the systemic circulation should be accurately known and (ii) the terminal phase during which V_{area} is computable should be a pure elimination phase.

When V_{area} is computed after extra-vascular drug administration and when the amount of drug that gains access to the systemic circulation is unknown, what is actually estimated is V_{area}/F, not V_{area} (Eqn 12):

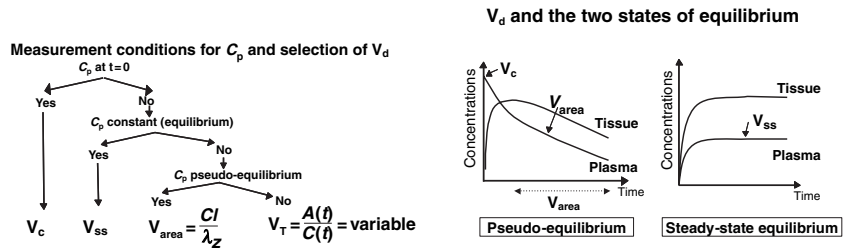


Fig. 4. The different volumes of distribution (V_d). The left panel indicates the appropriate V_d to consider (V_c, V_{area} or V_{ss}) when computing a drug amount in the body from a snapshot plasma concentration (Cp) regarding the state of drug distribution, i.e. at time 0, in equilibrium conditions or at pseudo-equilibrium conditions. The right panel explains the difference between a pseudo-equilibrium state of distribution (e.g. after an i.v. bolus drug administration) and an equilibrium condition (e.g. during an i.v. infusion). V_{area} is the appropriate V_d to consider during the terminal phase of a pseudo-equilibrium, whereas V_{ss} is the appropriate V_d to consider under steady-state conditions. During the distribution phase, V_d is not a parameter but a time dependent variable.

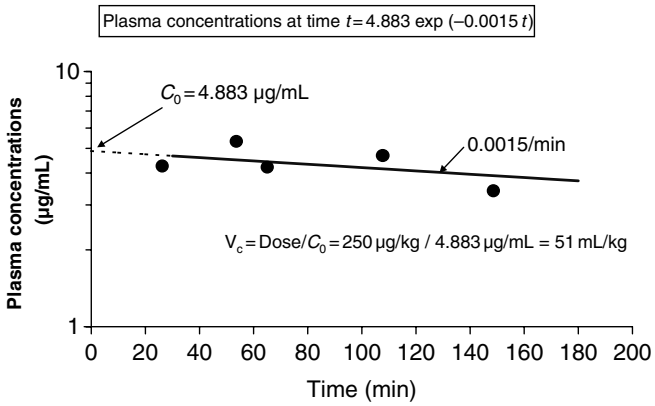


Fig. 5. The use of V_c to determine plasma volume. Evans blue (EB) is a dye classically used to determine plasma volume. A given amount of EB is administered by i.v. injection (e.g. 250 µg/kg) and after a delay of 20 min (*i.e* . after EB blood mixing), the disposition curve is evaluated from 20 to 180 min, the decay being mono-exponential. The plasma volume is estimated by V_c, the latter being estimated from the intercept [initial concentration (C_0) obtained by extrapolating the fitted curve to time 0]. The curve shown in this figure was simulated with the equation proposed by Wamberg *et al.* (2002) in dogs.

$$\frac{V_{area}}{F} = \frac{\text{Dose}}{AUC} \times \frac{1}{\lambda_z} \qquad (12)$$

where F is the bioavailability factor from 0 to 1. Equation 12 is equivalent to Eqn 11 only if $F = 1$.

If F is unknown (no i.v. study), V_{area}/F is at best of little value because V_{area}/F cannot be used to compute the actual amount of drug in the body after an extra-vascular administration. At worst, V_{area}/F may be advocated incorrectly to discuss the extent of drug distribution that will appear greatest while the bioavailability decreases. If one wishes to compute the residual amount of drug in the body after extra-vascular administration, the appropriate V_{area} is the one that has been computed by i.v. route.

Another, poorly appreciated requirement to compute a V_{area} is a terminal phase which is actually a pure elimination phase (in order to fulfil the assumption of distribution pseudo-equilibrium conditions in which the V_{area} has been defined). In this situation the computation of a V_{area}/F becomes wholly incorrect if the terminal phase actually corresponds to an absorption phase (flip-flop). Under these conditions, V_{area}/F can become very large as λ_z becomes small (see Eqn 12 and Fig. 8 for an example). This unacceptable computation and incorrect interpretation of V_{area} is frequently encountered in veterinary publications for avermectins and long-acting formulations of antibiotics. For example, Payne *et al.* (2002), compiled pharmacokinetic parameters reported in the literature for oxytetracycline in the goat. For long-acting formulations administered by the IM route, V_{ss} (or V_{area}) were considerably larger (4.1–11.9 L/kg) than V_{ss} (or V_{area}) reported after i.v. administration (0.5–3 L/kg).

STEADY-STATE VOLUME OF DISTRIBUTION (V_{SS})

As V_{area} relies on total body clearance, V_{area} is not an appropriate V_d in those situations for which clearance is null or apparently null. This is the case during i.v. infusion, once the steady-state condition has been reached and the rate of drug input exactly compensates for the rate of drug elimination. Under these conditions, the system behaves equivalently to a closed system (no input and no output), i.e. as having a null clearance.

In this circumstance, the use of V_{area} overestimates the total amount of drug in the body and the appropriate V_d to be selected will be the so-called V_{ss} with (Eqn 13):

$$V_{ss} = \frac{\text{Amount of drug in the body in equilibrium conditions}}{\text{Steady-state plasma concentrations } (C_{ss})} \qquad (13)$$

V_{ss} is a clearance independent volume of distribution that is used to calculate the drug amount in the body under equilibrium conditions, i.e. during a drug i.v. infusion and also during multiple drug administration once the steady-state conditions are achieved.

V_{ss} can be derived using different approaches (compartmental, statistical moments, …). For a classical mammillary compartmental model, V_{ss} is given by Eqn 14:

$$V_{ss} = V_c \left(1 + \sum_{j=2}^{n} \frac{K_{1j}}{K_{j1}}\right) \qquad (14)$$

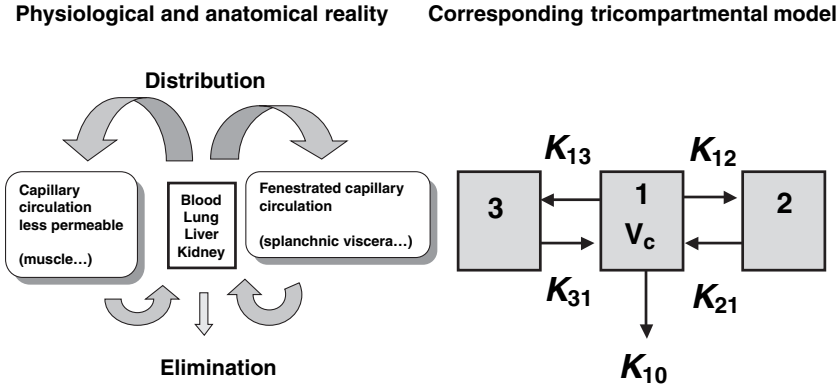


Fig. 6. Compartmental model and the volume of distribution of the central compartment. The figure shows the correspondence between a tricompartmental model (right) vs. the physiological and anatomical reality (left). The classical 2 or 3 compartmental mammillary models are a simplistic representation of the body in 2 or 3 well-stirred compartments. The mammillary topography is due to the anatomy of the cardiovascular system, which irrigates different organs in a parallel pathway (rather than sequentially). The central compartment corresponds to blood and all organs, which are in rapid equilibrium with blood (lungs, kidney and liver). The kidney and liver being the two most important clearing organs, drug elimination occurs from the central compartment (according to a first-order rate constant noted K_{10}), and the volume of the central compartment (V_c) can be viewed as the apparent space from which drug elimination occurs. It is the reason why body clearance can be estimated by the product of K_{10} and V_c. The peripheral compartment corresponds to organs for which the rate of equilibrium with blood is slower, the number of required peripheral compartments being indicated by the data itself. It should be noted that $K_{12}V_c$ and $K_{13}V_c$ are clearances of drug distribution for the shallow and deep peripheral compartment respectively, and they can be interpreted in terms of local organ blood flow.

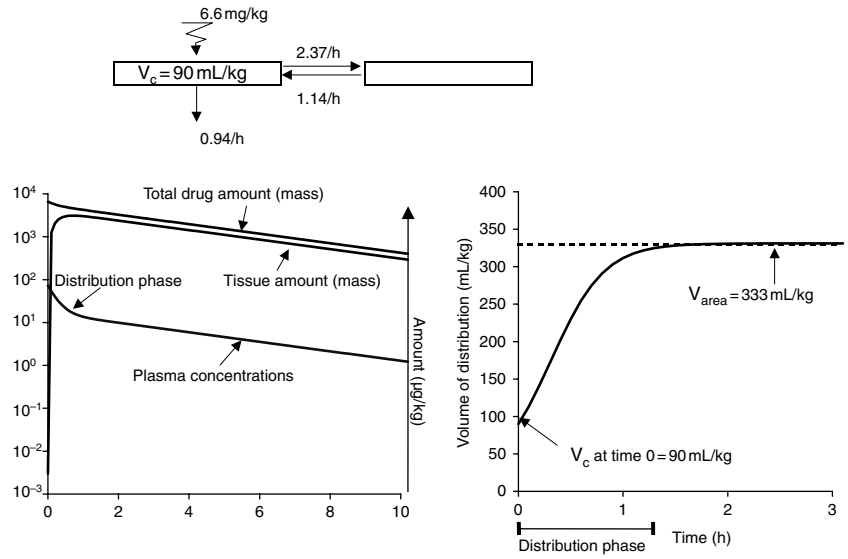


Fig. 7. Time development of the volume of distribution and distribution phases for a hypothetical drug. Data were simulated with parameters given for the represented bi-compartmental model. Left panel: during the distribution phase, drug plasma concentrations decrease more rapidly than the total amount of drug in the body because the decrease of drug plasma concentration is mainly because of the drug partition between vascular and extra-vascular spaces, and not to irreversible drug elimination. According to the definition of V_d, the V_d of the drug during the distribution phase should progressively increase to remain the proportionality factor between plasma concentrations and the total amount of drug (see right panel). And also during the distribution phase, V_d is a time dependent variable starting from an initial volume of distribution (V_c = 90 mL/kg) until it reaches an asymptotic value termed V_{area} (V_{area} = 333 mL/kg). V_{area} is a parameter because during the elimination phase, the ratio between the plasma concentration and the amount of drug in the body is a constant.

where K_{1j} and K_{j1} represent the distributional rate constants such as K_{12}, K_{21}, K_{13}, K_{31}, etc. of the general mammillary model.

V_{ss} can also be derived using the statistical moments approach described by Benet and Galeazzi (1979) (Eqn 15):

$$V_{ss} = \frac{\text{Dose}_{\text{i.v.}} \times AUMC}{(AUC)^2} = Cl \times MRT \qquad (15)$$

where *AUMC* is the area under the first moment of the disposition curve, *Cl* the plasma clearance, and *MRT* the mean residence time in the system.

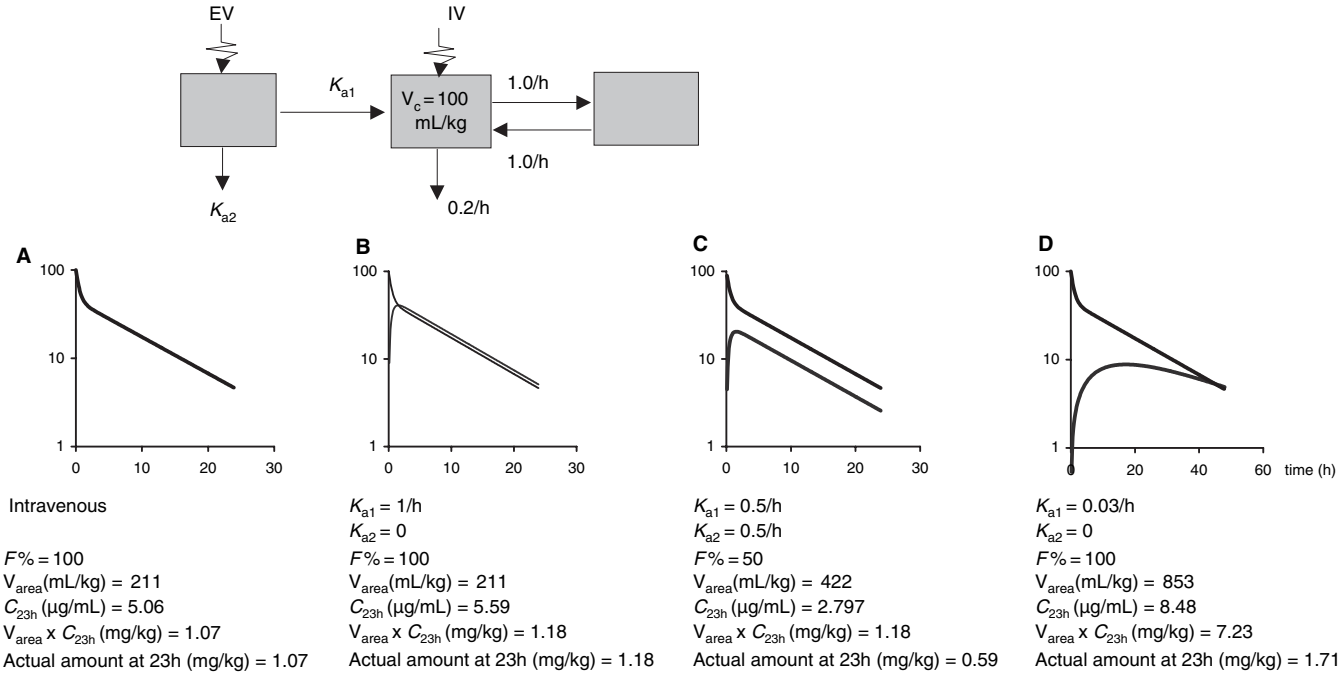


Fig. 8. V_{area} and the extra-vascular route of administration: influence of bioavailability and incorrect computation of V_{area} in presence of a flip-flop. Disposition curves corresponding to a classical bi-compartmental model were simulated after an intravenous (i.v.; panel A) or extra-vascular (EV; panels B, C and D) route of administration (dose 10 mg/kg). For the EV administration, three different sets of K_{a1} (first-order rate constant of drug transfer from the site of administration to the central compartment) and K_{a2} (which represents the irreversible drug loss from the site of administration) were selected to obtain a rapid and total (panel B), or a rapid but incomplete bioavailability (panel C), and a disposition curve characterized by a slow but complete absorption (flip-flop) (panel D).

V_{area}, terminal half-life and plasma concentration at 23 h post-injection were estimated by classical curve fitting. The residual amount of drug in the body at 23 h post-administration was estimated by the product $V_{area} \times C_{23\,h}$ and compared to the actual value. It can be seen that the true V_{area}, as obtained by i.v. route (211 mL/kg), can be obtained from an EV administration only if bioavailability is total and the rate constant of drug absorption is rapid ($t_{1/2}$ abs = 0.693 h) in relation to drug elimination ($t_{1/2}$ elimination = 7.3 h) (panel B). For the same apparent rate of drug absorption ($t_{1/2}$ abs = 0.693 h) but for an incomplete bioavailability (panel C), V_{area} is overestimated (422 mL/kg) because what is actually estimated is V_{area}/F (here $F = 50\%$ because $K_{a1} = K_{a2}$). Using the term V_{area}/F to estimate the residual amount of drug at 23 h post injection leads to overestimation (here twofold) of the true residual amount. In this situation, the appropriate V_{area} to consider is that calculated by the i.v. route. For panel D, the terminal half-life was estimated to be 31 h, a much longer half-life than after i.v. administration (7.3 h) indicating a flip-flop. V_{area} was estimated to be 850 mL/kg, which leads to estimate erroneously the residual amount of drug in the body (plasma and tissue) as 7.23 mg/kg. Actually, the drug amount remaining in the body (plasma and tissue) was only 1.71 mg/kg, whilst the residual amount at the injection site was 5 mg/kg. It should be noted that using V_{area} computed by the i.v. route (211 mL/kg), the residual amount of drug in the body (absorbed but not eliminated) is estimated to be 1.78 mg/kg, which is rather close to the true value (1.71 mg/kg).

The main assumptions for developing Eqn 15 are that the system is linear and that drug elimination takes place from the drug sampling site. The advantage of this method is that it does not require a curve fitting procedure but only a trapezoidal method.

V_{ss} can also be computed during a multiple dosing regimen, but in this situation Eqn 15 will overestimate the true V_{ss} and corrections are required (see Bauer & Gibaldi, 1983; Smith & Schentag, 1984). When data are obtained in steady-state conditions, V_{ss} is given by Eqn 16:

$$V_{ss}(\text{computed in steady-state conditions}) = \text{Dose}\left[\frac{(AUMC_{ss})_0^{\tau} + \tau(AUC_{ss})_{\tau}^{\infty}}{\left[(AUC_{ss})_0^{\tau}\right]^2}\right] \quad (16)$$

where τ, $(AUMC_{ss})_0^{\tau}$, $(AUC_{ss})_0^{\tau}$ and $(AUC_{ss})_{\tau}^{\infty}$ are the dosing interval, the area under the first moment curve within a dosing interval at steady-state, the area under the plasma concentration time curve within a dosing interval at steady-state, and the area under the plasma concentration curve from the last dose to infinity, respectively.

DIFFERENCES BETWEEN V_{AREA} AND V_{SS}

For all drugs, V_{area} is higher than V_{ss} but generally, the difference remains small. The difference between V_{area} and V_{ss} can be very large, however, if a large fraction of the drug is eliminated before reaching pseudo-equilibrium. This is the case for aminoglycosides when considering the very late terminal phase (Fig. 9). The difference between V_{area} and V_{ss} derives from the difference between pseudo-equilibrium and equilibrium conditions. In pseudo-equilibrium conditions, plasma drug concentrations decrease because the drug is continually

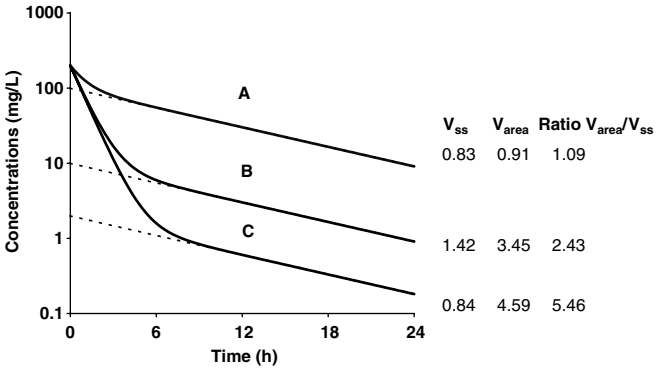


Fig. 9. Difference between the numerical value of V_{ss} and V_{area}. Three disposition curves (A, B, and C) corresponding to a bi-exponential equation with the same half-life of distribution (0.693 h) and the same half-life of elimination (6.93 h) were simulated for different sets of intercept (μg/mL) (Y_1 for the initial phase and Y_2 for the terminal phase with $Y_1 + Y_2 = 200$). For Curve A, $Y_1 = Y_2 = 100$. For curve B, $Y_1 = 190$ and $Y_2 = 10$. For curve C, $Y_1 = 198$ and $Y_2 = 2$. For each curve, V_{ss} and V_{area} were computed; it can be seen that when a large fraction of the dose has been eliminated during the initial phase (curve C), then V_{area} is much higher than V_{ss}. In contrast, if a minimal amount of drug is eliminated during the distribution phase, the numerical values of the two volumes of distribution are relatively similar.

removed from plasma at a rate proportional to plasma clearance (see Eqn 4). In contrast, in equilibrium conditions, plasma concentration is constant because the rate of drug elimination is compensated by the rate of drug input in the body (clearance is apparently null). Thus, all things being equal (i.e. for the same total amount of drug in the body), plasma concentration will be systematically lower in pseudo-equilibrium conditions than in equilibrium conditions. Therefore, when establishing the correlation between the same amount of drug in the body with plasma concentration, the proportionality constant should be higher in the pseudo-equilibrium state (V_{area}) than in the equilibrium state (V_{ss}), and V_{ss} can be viewed as the limit of V_{area} when the clearance tends towards zero.

The impact of clearance on V_{area} explains why V_{area} decreases when renal insufficiency exists. It would be erroneous to explain this decrease as an altered drug distribution. For instance, the pharmacokinetics of gentamicin was investigated in the horse before and after the occurrence of nephrotoxicity. V_{area} was reduced by 36%, i.e. a reduction proportional to that of body clearance (40%) whereas, as expected, the reduction of V_{ss} was more limited (19%) (Riviere *et al.*, 1983).

CLINICAL USE OF VOLUMES OF DISTRIBUTION (V_C, V_{AREA} AND V_{SS})

Volumes of distribution were conceived to compute drug amount and loading dose. Conversely, V_c and V_{ss} can be used to predict critical concentrations. In practice, V_c is seldom used. It can be useful to predict the initial maximum concentration for an i.v. bolus administration (e.g. in anaesthesiology), and to anticipate possible side-effects when a loading dose is rapidly administered with a possible initial high peak plasma concentration, making it desirable to divide the loading dose. Another application of V_c is to estimate the plasma volume when using a compound which is restricted to plasma such as Evans blue (Fig. 5) (Wamberg *et al.*, 2002).

V_{area} (not V_{area}/F) is used to estimate the residual amount of drug in the body when the drug decreases according to its elimination phase. In a mass balance trial, V_{area} can be helpful to predict how much drug remains to be excreted, or the overall amount of drug residue in the body.

V_{ss} is the most useful V_d because it allows computation of a loading dose (Eqn 17):

$$\text{Loading dose} = \frac{V_{ss} \times C_{ss}}{F} \qquad (17)$$

where C_{ss} is the expected (desired) plasma concentration at steady-state and F (0–1), is the bioavailability factor. A loading dose can be required for some drugs when it is desirable to reach immediately or rapidly the C_{ss} (e.g. under life threatening conditions for anti-coagulant, anti-epileptic, anti-arrhythmic, antimicrobial therapy,…) and when the drug has a long terminal half-life. As discussed in the companion paper on half-life, the time required to reach steady-state conditions is approximately 4–5 times the terminal half-life, regardless of the frequency of the drug administration. For instance, potassium bromide, for the therapy of epilepsy in dog, has a very long terminal half-life (from 12 to 20 days). Steady-state condition (90% of C_{ss}) is only reached after approximately 2 months (March *et al.*, 2002) when administering the daily maintenance dose, a delay during which the patient is underexposed with regard to the final targeted C_{ss}. For potassium bromide, V_{area} (close to V_{ss} because the body clearance is very low) was estimated to be 0.45 ± 0.07 L/kg (Trepanier & Babish, 1995) and an i.v. loading dose of 450 mg/kg has been suggested to immediately reach a minimal therapeutic bromide plasma concentration (1000 mg/L) (Trepanier & Babish, 1995). This loading dose is about 15–20 times higher than the oral maintenance dose (20–30 mg/kg BID) (March *et al.*, 2002) and caution must be exercised in regard to the risk of achieving transitory excessive (toxic) concentrations. Practically, the oral loading dose of 400–600 mg/kg is divided into four doses, mixed with food, and given over a 24-h period of time to reduce nausea and vomiting (Dowling, 1999).

In emergency situations, an i.v. loading dose (generally followed by an infusion to maintain C_{ss}) can be appropriate to immediately reach the C_{ss}, as is the case for the anti-arrhythmic drug lidocaine (Nunes de Moraes *et al.*, 1998).

Figure 10 summarizes the main difference between the maintenance and loading doses.

V_{ss} can also be used to predict the fluctuation of plasma concentrations during a dosage interval for an intermittent dosage regimen. In the equilibrium condition, the plasma concentration is decreased from the peak (maximum) plasma

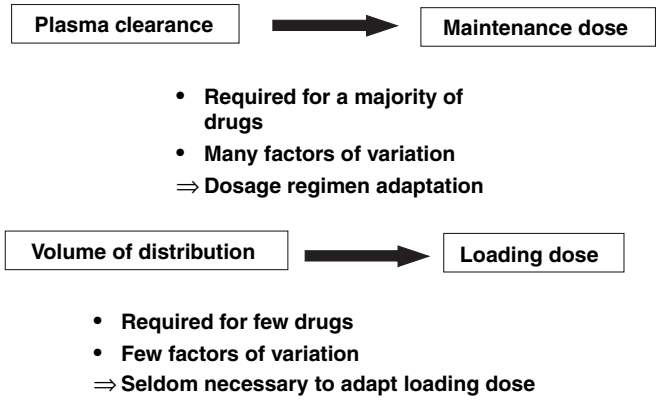


Fig. 10. Features of maintenance vs. loading doses.

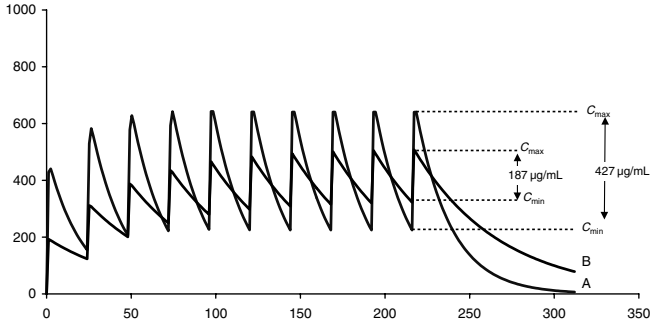


Fig. 11. Volume of distribution (V_{ss}) and plasma concentration fluctuations at steady-state. This figure illustrates that the amplitude of plasma drug concentration fluctuation (the difference between the C_{max} and the C_{min}) is influenced by the extent of the drug distribution. Simulated curves correspond to two different drugs (A and B) having the same plasma clearance, the same rate constant of absorption (1 h^{-1}) but different V_{ss} (0.2 L/kg and 0.5 L/kg for drugs A and B, respectively). The same dosage regimen (Dose 100 mg/kg, dosing interval 24 h) has been simulated. At steady-state, the actual amplitude of fluctuation was 427 and 187 µg/mL for drugs A and B respectively, whereas Eqn 18 predicted 500 and 200 µg/mL (assuming $F = 100\%$). The difference between the actual and predicted value is due to the fact that Eqn 18 does not take into account that drug disposition follows multiexponential kinetics, and that the actual C_{max} and C_{min} are functions of a set of several exponential constants.

concentration (C_{max}) to a trough (minimum) concentration (C_{min}) (Fig. 11). The amplitude of the fluctuation can be approximated by (Eqn 18):

$$C_{max} - C_{min} = \frac{F(\text{Dose})}{V_{ss}} \quad (18)$$

Therefore, the larger the V_{ss}, the smaller the fluctuation and vice versa. Thus, for drugs having a narrow therapeutic window, the dose and dosing interval can be appropriately selected to minimize plasma concentration fluctuations.

Finally, V_d can be used to anticipate the usefulness of dialysis for the treatment of drug intoxication in emergency. Indeed, comparison of the V_d to the plasma volume indicates if the drug present in the body is mainly located in the vascular or extra-vascular spaces, then dialysis is indicated in the former case.

THE INCORRECT USE OF VOLUME OF DISTRIBUTION TO EXPLAIN LEVELS OF PLASMA CONCENTRATION

Volumes of distribution are frequently used to discuss the extent of drug distribution. It should be emphasized again that V_d was not primarily designed for this purpose, and to say that average (C_{ss}) plasma concentrations are low because V_{ss} is large would imply rearranging implicitly and incorrectly the Eqn 1 into (Eqn 19):

$$C_{ss} = \frac{\text{Amount at equilibrium}}{V_{ss}} \quad (19)$$

This expression is not valid when conceptualizing dependency and functionality because it involves inter-relating the dependent variable (V_{ss}) and the independent variable (C_{ss}). In the framework of V_d, C_{ss} is the variable that controls V_{ss}.

Contrary to expected intuition, it is not V_{ss} which controls C_{ss} but the plasma clearance (Eqn 20):

$$C_{ss} \times \text{Dosing interval} = AUC_{ss} = \frac{F \times \text{Dose(maintenance)}}{\text{Clearance}} \quad (20)$$

Equation 20 also indicates that the total AUC is wholly independent from V_{ss}. *AUC* is controlled only by the plasma clearance, and V_d is associated only with the shape of the disposition curve and not its area.

A MINIMAL PHYSIOLOGICAL MODEL FOR THE INTERPRETATION AND PREDICTION OF A VOLUME OF DISTRIBUTION

If one wishes to discuss V_d in terms of drug repartition, it is necessary to have recourse to a physiological model. For this purpose, V_{ss} is the appropriate V_d.

Taking into account that only unbound drug is capable of entering and leaving the plasma and tissue (see Fig. 3), a minimal physiological model of V_{ss} can be established according to the principle of mass balance (Eqn 21):

$$V_{ss} = V_P + V_T \frac{fu_{,P}}{fu_{,T}} \quad (21)$$

where V_P is the plasma volume and V_T is the volume outside the plasma into which the drug distributes. In the first instance, V_T can be approximated by total body weight (Jusko & Chiang, 1982). $fu_{,P}$ is the fraction unbound in plasma, and $fu_{,T}$ is the fraction unbound outside plasma. The ratio $fu_{,P}/fu_{,T}$ can be seen as the overall drug partition coefficient (K_P) between plasma and other tissues (Eqn 22). Figure 12 presents a pictorial view of the minimal model for a V_d using a flask analogy to the body.

Equation 21 indicates that a drug which is largely bound to plasma protein ($fu_{,P}$ small) will tend to have a rather small V_d,

whereas a drug extensively bound to tissue ($fu_{,T}$ small) will generally have an apparent large V_d. Thus, $fu_{,T}$ and $fu_{,P}$ have opposite effects on V_d, and the extent of distribution in the body can be regarded as the result of a binding competition between plasma and tissue (Bickel & Gerny, 1980).

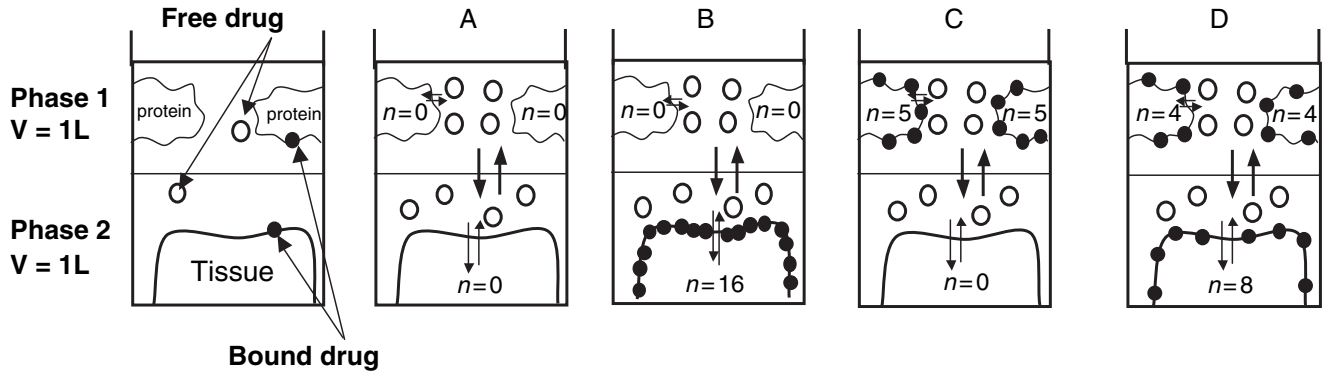
$fu_{,T}$, in contrast to $fu_{,P}$ is not easily measurable. Currently, there is evidence of a lack of correlation between $fu_{,P}$ and $fu_{,T}$, but the correlation between binding in different tissues (Fichtl *et al.*, 1991) suggests that this process may be governed by similar mechanisms whatever the tissues.

For a drug having a large V_{ss}, V_P in Eqn 21 can be neglected and V_d interpreted using Eqn 22:

$$V_{ss} = V_T \frac{fu_{,P}}{fu_{,T}} = V_T K_P \qquad (22)$$

where K_P is the overall partition coefficient and V_T is approximated by the body weight : 1 L/kg or 30 L for a 30 kg dog.

If V_{ss} is expressed in L/kg (i.e. if V_T is 1 L/kg), the numerical value of V_{ss} is equal to K_P. For example, the V_{ss} of chlorpromazine in the dog is 18.6 L/kg; using Eqn 22, K_P can therefore be



	A	B	C	D
$V_d = \frac{\text{Total amount of drug in the flask}}{\text{Phase 1 total concentration}}$	8/4 = 2 L	24/4 = 6 L	18/14 = 1.286 L	24/12 = 2 L
$V_d = \frac{\text{Total number of dots}}{\text{Number of dots in plasma}}$				
$fu_{,P} = \frac{\text{Free concentration phase 1}}{\text{Total concentration phase 1}}$	4/4 = 1	4/4 = 1	4/14 = 0.286	4/12 = 0.333
$fu_{,T} = \frac{\text{Free concentration phase 2}}{\text{Total concentration phase 2}}$	4/4 = 1	4/20 = 0.2	4/4 = 1	4/12 = 0.2333
$K_P = fu_{,P}/fu_{,T}$	1	5	0.286	1
Model $V_d = V_1 + V_2 \times \frac{fu_{,P}}{fu_{,T}}$	1 + 1 = 2L	1 + 1 × 5 = 6 L	1 + 1 × 0.286 = 1.286 L	1 + 1 × 1 = 2 L

Fig. 12. Models of volume of distribution using a flask analogy model. A hypothetical drug partition between two water phases (1 and 2) having the same volume (1 L) and being analogous to plasma (1) and tissue (2). The drug concentration is symbolized by a given number of dots. In phases 1 and 2 drug can be free (○) or bound (●) to protein, tissues… The system being in equilibrium, the free drug concentration is the same in phase 1 and phase 2 (symbolized here by 4 empty dots, in each water phase). In phase 1, drug can bind to protein leading to computation of a free fraction denoted $fu_{,P}$. Similarly, for the phase 2 representing the tissue compartment, the free fraction is denoted $fu_{,T}$.
The figure in each inset gives the number of solid dots fixed on plasma protein or tissue components. Using this analogous system, the definition of volume of distribution is applied:

$$V_d = \frac{\text{Total number of dots in the flask}}{\text{Total dot concentration in phase 1 of the flask}}$$

The V_d is also computed using a model of the form:

$$V_d = \text{volume of phase 1 (1 L)} + \text{volume of phase 2 (1 L)} \times \frac{fu_{,P}}{fu_{,T}}$$

where $fu_{,P}$ and $fu_{,T}$ are the number of free dots over the total number of dots in each phase. This model is the physiological model for a V_d (see Eqn 21 in the text). The ratio: $K_P = fu_{,P}/fu_{,T}$ is the partition coefficient of the system.

estimated to 18.6, K_P expressing the overall affinity of the body for the drug and V_{ss} expresses the capacitance property of the body for that drug.

WHY AND HOW TO PREDICT V_{SS} IN THE EARLY STAGES OF DRUG DEVELOPMENT

Only drugs in the vascular system can be presented to the clearing organs for elimination, and volume of distribution is one of the determinants of the terminal half-life (Eqn 28 *vide infra*). In early drug development, the V_d of different analytes can be considered for its impact on terminal half-life and hence the duration of drug action : the higher the V_d the longer the half-life.

For different competing similar drugs, V_d can be predicted *in silico*, using different regression models including drug lipophilicity and plasma protein binding as input parameters, two properties which are available early in drug development (including *in silico* computation) (Poulin & Theil, 2002).

V_d can also be predicted by some *in vitro* investigations to estimate K_P (Eqn 22). The two main contributors to V_T are muscle (about 40% of the BW) and fat (from 10 to 40% of BW depending on age and feeding regimen) and according to Bjorkman (2002) it is possible to predict the V_{ss} for a drug from knowledge of only two K_P (K_P for fat and muscle). The muscle K_P can be used to represent all lean tissues including carcass but the fat K_P seems to predict better the one of lung for basic drugs.

Another means of predicting a V_d is to extrapolate V_{ss} from one species to another. For a series of basic drugs, it was shown that the term $V_T/ fu_{,T}$ was relatively similar in different species, allowing prediction of a V_d in a new species by determining only $fu_{,P}$ (Sawada *et al.*, 1984).

THE POTENTIALLY MISLEADING USE OF VOLUME OF DISTRIBUTION TO ANTICIPATE THE LEVEL OF TISSUE DRUG CONCENTRATIONS

It is often quoted 'a high tissue concentration should be expected for that drug by comparison with another one because the difference between the volumes of distribution is very large'. Actually, a large difference in V_d can only reflect a small change in tissue concentrations. For instance, considering a drug having a V_d of 1 L/kg with 90% of the drug in tissue, and a drug having a V_d of 10 L/kg with 99% of the drug in tissue; there is a difference of only 10% between the two drugs in terms of amount located in tissue.

THE RELATIONSHIP BETWEEN V_{SS} AND THE AMOUNT OF DRUG IN THE BODY IN TERMS OF DRUG BINDING

The total amount of drug in the body is given by Eqn 23:

$$\text{Total amount of drug in the body} = V_d \times \text{Plasma concentration}_{(\text{total})} \quad (23)$$

Replacing V_d by Eqn 22:

$$\text{Total amount of drug in the body} = V_T \times \frac{fu_{,P}}{fu_{,T}} \times \text{Plasma concentration}_{(\text{total})} \quad (24)$$

Considering that $fu_{,P} \times$ Plasma concentration is equal to the free drug plasma concentration (C_{free}) Eqn 24 can be rewritten (Eqn 25):

$$\text{Total amount of drug in the body} = V_T \times \frac{1}{fu_{,T}} \times C_{\text{free}} \quad (25)$$

Inspection of Eqn 25 shows that the total amount of drug in the body is not influenced by $fu_{,P}$ (i.e. drug binding to plasma protein) and that no adjustment of the loading dose is required when $fu_{,P}$ is altered.

THE USE OF TOTAL TISSUE CONCENTRATION TO DISCUSS THE LOCAL FREE REGIONAL EXTRACELLULAR CONCENTRATIONS

When interpreting a large V_{ss} or a high total tissue concentration, it should be stressed that it is the free drug concentration in extracellular fluid which controls the total tissue concentration and not the inverse. For instance, an increase in $fu_{,T}$ will result in a proportional decrease of tissular total concentration but will have no effect on free concentration in the area or in any other part of the body (Gibaldi & Koup, 1981).

Actually, as for the free plasma concentration, the free (local) extracellular concentration is controlled only by the free plasma clearance (i.e. the intrinsic clearance for drugs having a low extraction ratio), and not by the total tissue concentration. This explains why a high total tissue concentration does not guarantee an appropriate extracellular drug concentration (e.g. for an antibiotic), and that a total tissue concentration has no relevant intrinsic therapeutic meaning, especially when the biophase is located in extracellular water of the target tissue (which is the case for most pathogens of veterinary interest).

The relevant concentration when the biophase is located in extracellular fluid of a given tissue is the free plasma concentration, which is in equilibrium with the free concentration in extra-vascular water.

INTERPRETATION OF SMALL VOLUME OF DISTRIBUTION

Equation 21 (or Equation 22) is not satisfactory for the physiological interpretation of small values of V_{ss} (i.e. $V_d < 0.2$ L/kg).

Oie and Tozer (1979), proposed a model for small volumes of distribution having three physiological spaces and for a man of 70 kg BW they provided the following equation (Eqn 26):

$$V(L) = 7.5 + 7.5 fu_{,P} + V_T \frac{fu_{,P}}{fu_{,T}} \quad (26)$$

where V_T at variance with Eqn 22, is assimilated into the volume of intracellular water (24 L in man). The same equation scaled in body weight units is (Eqn 27):

$$V(L/kg) = 0.107 + 0.107 fu_{,P} + 0.343 \frac{fu_{,P}}{fu_{,T}} \qquad (27)$$

Using this model, the smallest V_d is no longer the plasma volume (3 L for a man or 0.04 L/kg) but 7.5 L for a man or (0.11 L/kg), and it corresponds to a drug which will be integrally bound to plasma protein ($fu_{,P} = 0$). The minimal volume is higher than the plasma volume because plasma proteins also diffuse out of the vascular space (extracellular fluid) and return through the lymphatic system to plasma. Thus the minimal V_d corresponds to the apparent distribution space of the circulating and recirculating plasma proteins. If a lower V_d is computed, it is likely that it has been evaluated before the drug has been totally distributed through the protein circulation space. For a drug restricted to the extracellular fluid and not bound to plasma protein ($fu_{,P} = 1$), the apparent volume of distribution is 15 L for a man (or about 0.22 L/kg), i.e. equivalent to the extra-vascular water space (e.g. the inulin V_d). Using this model, the repartitioning of drug (free and bound) in the body can be predicted from $fu_{,P}$ and V_{ss} (Rowland & Tozer, 1995). For many pathogens of veterinary interest, the biophase is the extracellular water space and, all other things being equal, the most appropriate antibiotic is the one for which the highest percentage of drug amount in the body is located in this phase as free drug. Using this criterion, the ideal drug has a V_d of 0.68 L/kg and is 100% free [29% of drug (free) located in the extracellular fluid]. For a drug bound at 50%, the ideal V_d is 0.42 L/kg (24% of free drug is located in the biophase). For a drug bound at 90%, the ideal V_d is 0.2 L/kg (10% of free drug is located in the biophase). Figure 13 gives the percentage of the drug which is located in the extracellular fluid (as free drug) for different volumes of distribution and different binding to plasma protein.

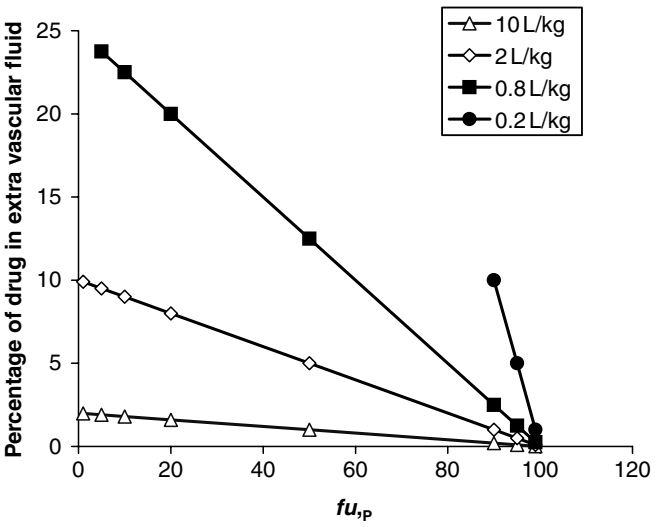


Fig. 13. Volume of distribution, extent of protein binding and drug location in the extracellular fluid outside plasma. The values were calculated using the equation given by Rowland and Tozer (1995) (pp. 497) for hypothetical drugs having volumes of distribution of 0.2, 0.8, 2 and 10 L/kg and for each V_d, for different extent of drug binding (0–100%). Inspection of this figure shows that drug binding to plasma protein has a strong influence on the fraction of drug located in the extracellular fluid outside plasma, when V_d is small, but marginal when V_d is large.

TERMINAL HALF-LIFE AND MODEL OF VOLUME OF DISTRIBUTION

After an i.v. bolus administration, the terminal half-life ($t_{1/2}$) is a hybrid parameter which can be interpreted in terms of both clearance and volume of distribution according to Eqn 28:

$$t_{1/2} = \left(\frac{0.693}{\text{Clearance}}\right) V_{area} \qquad (28)$$

A long $t_{1/2}$ may be associated either with a large V_d or/and a low clearance. The influence of V_d (or more exactly of the drug distribution) is because of the fact that only drug in the vascular system can be presented to the eliminating organs (kidney and liver). The larger the V_d, the lower the fraction of the drug that can be eliminated over time and the longer the half-life.

Incorporating the model of V_d (Eqn 22) in Eqn 28 allows for a more advanced interpretation of $t_{1/2}$ (Eqn 29):

$$t_{1/2} = \frac{0.693}{\text{Clearance}} \left(V_T \frac{fu_{,P}}{fu_{,T}}\right) \qquad (29)$$

For a drug having a low extraction ratio, the rate of elimination is proportional to the free plasma concentration (filtration in the kidney, hepatic clearance). Therefore, the body clearance is equal to $fu_{,P} \times Cl_{free}$ with Cl_{free}, the clearance of the free drug. Incorporating $fu_{,P} \times Cl_{free}$ in Eqn 29 gives (Eqn 30):

$$t_{1/2} = \left[\frac{0.693}{fu_{,P} \times Cl_{free}}\right] V_T \frac{fu_{,P}}{fu_{,T}} \qquad (30)$$

And after simplification (i.e. deleting $fu_{,P}$) (Eqn 31):

$$t_{1/2} = \left(\frac{0.693}{Cl_{free}}\right)\left(\frac{V_T}{fu_{,T}}\right) \qquad (31)$$

Equation 31 indicates that $t_{1/2}$ for a restricted-clearance drug cannot be influenced by $fu_{,P}$, i.e. plasma binding, but is dependent on tissue binding (i.e. $fu_{,T}$). In contrast, for drugs having a high extraction ratio (i.e. a clearance which is flow dependent), $t_{1/2}$ will be influenced by $fu_{,P}$.

Table 1 gives the terminal half-life, clearance and volume of distribution of ivermectin, doramectin and moxidectin in cattle. It is clear that the long terminal half-life of moxidectin is mainly due to its large V_d, which in turn can be interpreted in terms of drug binding to plasma and tissues.

Table 1. Volumes of distribution, clearance and terminal half-life for ivermectin, doramectin and moxidectin in cattle. The parameters were obtained after the administration of the three drugs as a single cocktail in eight cattle (A. Bousquet-Mélou *et al.*, unpubl. results)

	Clearance (mL/day/kg)	V_c	V_{ss} (L/kg)	V_{area}	$t_{1/2}$ (days)
Ivermectin	347	0.053	2.72	4.20	7.8
Doramectin	185	0.55	3.26	3.99	14.2
Moxidectin	636	0.033	14.9	20.8	21.5

Despite the highest total body clearance, moxidectin has the longest terminal half-life as a result of its very large volume of distribution.

For antibiotics, it is often desirable to have a long terminal half-life in order to have a single-dose treatment to optimize compliance, but the strategy for achieving this goal can be very different between the case of long-acting formulations, for which the rate of drug absorption controls the terminal half-life (flip-flop), and the strategy for a drug with a large V_d (e.g. macrolides). The limitation of the first strategy is the presence of residues at the site of injection. The limitation of the second is to have very large total tissue concentrations compared to lower extra-vascular (water) concentrations, i.e. into the body compartment, which is the most frequent biophase for pathogens of veterinary interest. Currently, it seems that the latter strategy is successful for macrolides for which efficacy can be obtained with plasma concentrations lower than *in vitro* minimum inhibitory concentration (MIC).

CONCLUSION

Clearance is the relevant parameter to compute the maintenance dose, whilst V_{ss} is the pharmacokinetic parameter to compute a loading dose.

Using an appropriate model for the interpretation/prediction, the volume of distribution can be predicted in drug development and can assist drug companies in designing drugs with a long terminal half-life, and in anticipating a persistence of drug residues.

REFERENCES

Bauer, L.A. & Gibaldi, M. (1983) Computation of model-independent pharmacokinetic parameters during multiple dosing. *Journal of Pharmaceutical Sciences*, **72,** 978–979.

Benet, L.Z. & Galeazzi, R.L. (1979) Noncompartmental determination of the steady-state volume of distribution. *Journal of Pharmaceutical Sciences*, **68,** 1071–1074.

Bickel, M.H. & Gerny, R. (1980) Drug distribution as a function of binding competition. Experiments with the distribution dialysis technique. *Journal of Pharmacy and Pharmacology*, **32,** 669–674.

Bjorkman, S. (2002) Prediction of the volume of distribution of a drug: which tissue-plasma partition coefficients are needed? *Journal of Pharmacy and Pharmacology*, **54,** 1237–1245.

Dowling, P.M. (1999) Update on therapy of canine epilepsy. *Canadian Veterinary Journal*, **40,** 595–598.

Fichtl, B., v. Nieciecki, A. & Walter, K. (1991) Tissue binding versus plasma binding of drugs: general principles and pharmacokinetic consequences. In *Drug Research*. Ed. Bernard, T. pp. 118–166. Academic Press, London, UK.

Gibaldi, M. & Koup, J.R. (1981) Pharmacokinetic concepts – drug binding, apparent volume of distribution and clearance. *European Journal of Clinical Pharmacology*, **20,** 299–305.

Jusko, W.J. & Chiang, S.T. (1982) Distribution volume related to body weight and protein binding. *Journal of Pharmaceutical Sciences*, **71,** 469–470.

March, P.A., Podell, M. & Sams, R.A. (2002) Pharmacokinetics and toxicity of bromide following high-dose oral potassium bromide administration in healthy Beagles. *Journal of Veterinary Pharmacology and Therapeutics*, **25,** 425–432.

Nunes de Moraes, A., Dyson, D.H., O'Grady, M.R., McDonell, W.N. & Holmberg, D.L. (1998) Plasma concentrations and cardiovascular influence of lidocaine infusions during isoflurane anesthesia in healthy dogs and dogs with subaortic stenosis. *Veterinary Surgery*, **27,** 486–497.

Oie, S. & Tozer, T.N. (1979) Effect of altered plasma protein binding on apparent volume of distribution. *Journal of Pharmaceutical Sciences*, **68,** 1203–1205.

Payne, M.A., Babish, J.G., Bulgin, M., Lane, M., Wetzlich, S. & Craigmill, A.L. (2002) Serum pharmacokinetics and tissue and milk residues of oxytetracycline in goats following a single intramuscular injection of a long-acting preparation and milk residues following a single subcutaneous injection. *Journal of Veterinary Pharmacology and Therapeutics*, **25,** 25–32.

Poulin, P. & Theil, F.P. (2002) Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. *Journal of Pharmaceutical Sciences*, **91,** 129–156.

Riviere, J.E., Coppoc, G.L., Hinsman, E.J., Carlton, W.W. & Traver, D.S. (1983) Species dependent gentamicin pharmacokinetics and nephrotoxicity in the young horse. *Fundamental and Applied Toxicology*, **3,** 448–457.

Rowland, M. & Tozer, T.N. (1995) *Clinical Pharmacokinetics. Concepts and Applications*, 3rd edn. Williams and Wilkins, London, UK.

Sawada, Y., Hanano, M., Sugiyama, Y., Harashima, H. & Iga, T. (1984) Prediction of the volumes of distribution of basic drugs in humans based on data from animals. *Journal of Pharmacokinetics and Biopharmaceutics*, **12,** 587–596.

Smith, I.L. & Schentag, J.J. (1984) Noncompartmental determination of the steady-state volume of distribution during multiple dosing. *Journal of Pharmaceutical Sciences*, **73,** 281–282.

Trepanier, L.A. & Babish, J.G. (1995) Pharmacokinetic properties of bromide in dogs after the intravenous and oral administration of single doses. *Research in Veterinary Science*, **58,** 248–251.

Wamberg, S., Sandgaard, N.C. & Bie, P. (2002) Simultaneous determination of total body water and plasma volume in conscious dogs by the indicator dilution principle. *Journal of Nutrition*, **132,** 1711S–1713S.