The cell envelope, and particularly the outer membrane, of Gram-negative bacteria is known to change in response to changes in the growth environment (8, 11, 12). It is therefore likely that these changes in resistance may reflect changes in the cell envelope which either prevent access of the drug to the site of action or alter the site of action such that the drugs show decreased activity. Little is known about the cell envelope of *P. cepacia*. The lipopolysaccharide is atypical, not containing 2-keto-3-deoxyoctonate and with several quantitative differences in the sugar moieties (13). The cellular fatty acid compositions (mostly derived from the envelope phospholipids) of *P. cepacia* and *Pseudomonas aeruginosa* differ in that the former has proportionately more cyclopropane fatty acids than does the latter (14).

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COMMUNICATIONS

Mean Hepatic Transit Time in the Determination of Mean Absorption Time

Keyphrases □ Mean hepatic transit time—determination of mean absorption time, pharmacokinetics □ Mean absorption time—determination by mean hepatic transit time, pharmacokinetics □ Pharmacokinetics—mean hepatic transit time, mean absorption time, mean residence time

To the Editor:

In recent studies the statistical moment theory has been employed to estimate mean absorption time (MAT) of drugs (1–5). The MAT has been defined as the mean time of a molecule (1) or the mean residence time (MRT) of all molecules (2) from a dosage form (such as solution or tablet) spent at the input site (GI lumen in the case of oral administration) before being absorbed into the general circulation. The MAT after oral administration has been calculated based on the following equation (1–5):

$$MAT = MRT_{po} - MRT_{iv} \qquad \text{(Eq. 1)}$$

where MRT_{po} is the MRT of the orally absorbed drug molecules in the body, and MRT_{iv} is the MRT of intravenously (usually from a peripheral vein in the leg or arm) administered drug molecules in the body. Both plasma and urinary excretion data have been proposed to estimate the MRT_{po} and MRT_{iv}. When a solution dosage form is studied, the calculated MAT has been referred to as mean intrinsic absorption time (5). When both solution and solid dosage forms are evaluated, the difference in their MRT may be considered to equal the mean *in vivo* dissolution time (MDT) from the solid dosage form. This is based on the assumption that once released from the solid dosage form, it is subject to the same influence as the drug administered in solution (1–3).

The main purpose of this communication is to discuss a complication in using Eq. 1 to determine the MAT. Its potential significance in absorption rate calculations and hepatic clearance studies will also be briefly mentioned. In analogy to the above assumption requirement in the determination of MDT, use of Eq. 1 must also require that the orally absorbed drug is handled in the body exactly the same way as that administered intravenously. This demand apparently can not be met because the orally absorbed drug has to pass through the GI wall and then the liver before entering the heart, while the intravenously administered drug can be carried almost instantaneously from the injection site to the heart before being distributed to the rest of body. The mean time to pass through the GI wall and liver can be called mean GI wall transit time, MTT_{GI}, and mean hepatic transit time, MTT_h, respectively. The portal circulation between the GI wall and liver is extremely fast and can be ignored. Therefore, Eq. 1 can be modified to:

$$MAT = MRT_{po} - MRT_{iv} - MTT_{GI} - MTT_h \qquad \text{(Eq. 2)}$$

The MTT in a tissue or organ during a single passage can be determined directly by instantaneous injection of a compound into the affluent blood and monitoring of effluent blood concentration, C_{out}, under the single-pass nonrecirculating condition (6–8):

$$MTT = \frac{\int_0^\infty tC_{out}dt}{\int_0^\infty C_{out}dt} \qquad \text{(Eq. 3)}$$

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In linear pharmacokinetics it can also be estimated by (6–9):

$$MTT = V_{ss}/Q \qquad \text{(Eq. 4)}$$

where V_{ss} is the apparent (effective) steady-state volume of distribution of the tissue or organ for a given drug and Q is the blood flow rate through that tissue or organ. In view of the fast blood flow through the small section (with a limited tissue mass during each passage) of major GI absorption sites (usually in the small intestine) the MTT_{GI} may be expected to be relatively short or negligible for most drugs except those with extremely high binding property. Furthermore, the precise location and fraction absorbed at each location usually can not be ascertained. Therefore, under most circumstances the MTT_{GI} probably can be ignored.

The V_{ss} for the liver may be estimated by (10, 11):

$$V_{ss} = V_b + R_h V_h \qquad \text{(Eq. 5)}$$

where V_b is the volume of blood in the liver, R_h is the hepatic tissue–venous blood partition coefficient of drug, and V_h is the hepatic tissue volume (excluding blood). For a normal 70-kg human adult the V_b, V_h, and Q_h can be assumed to be 0.37, 1.5, and 1.58 liters/min, respectively (12, 13). Substitution of the above values into Eqs. 4 and 5 will result in the following equation for estimating the MTT_h for various drugs in human adults:

$$MTT_h \text{ in min} = (0.37 + 1.5R_h)/1.58 \qquad \text{(Eq. 6)}$$

The actual determination of R_h values in normal humans is extremely difficult or virtually impossible under practical circumstances. In view of the many reported successes in interspecies scaling in pharmacokinetic studies (11, 13–15) R_h values of several drugs in animals reported or estimated from the literature (15–18) will be used here for the purpose of illustration; as an approximation, the drug is also assumed to be instantaneously (19, 20) and evenly distributed in whole blood. The results of the calculation are summarized in Table I. The MTT_h obtained ranged from 0.52 min for tolbutamide to 977 min (16.3 hr) for chloroquine. The unusually long transit times estimated here for chloroquine and hydroxychloroquine (12.8 hr) during a single passage through the liver are somewhat of a surprise. These results indicate that for chloroquine and hydroxychloroquine it may take an average of more than 10 hr before they are transported from the liver by the blood stream or before they are metabolized by the liver following absorption. The MTT_h for the blood across the liver, on the other hand, can be estimated to be only 0.234 min (0.37/1.58) or 14 sec, which is only slightly higher than the values of 8.4 and 9.0 sec, reported earlier in the dog (7) and rat (21), respectively. It should be noted that the blood MTT across hepatic sinusoids, where diffusion of drug into hepatocytes for biotransformation and/or biliary excretion takes place, could be 36% shorter (7).

Table I—Mean Hepatic Transit Times (MTT_h) of Several Drugs in Humans Estimated Based on the Hepatic Tissue–Plasma Concentration Ratios (R_h) Obtained from Animal Studies

Drug[a]	Animal Species for R_h value	R_h	MTT_h (min)
Sulfobromophthalein (15)	Rat	7.0	6.9
Digoxin (15)	Dog	15.8	15.2
Dactinomycin (15)	Dog	32.2	30.8
Doxorubicin (15)	Rabbit	60.4	57.6[c]
Tolbutamide (16)	Rat	0.30	0.52
Chlorpheniramine (10, 17)[b]	Rabbit	31.4	30.0
Chloroquine (18)	Rat	1029.0	977.0[d]
Hydroxychloroquine (18)	Rat	811.0	770.0[d]

[a] Number in parentheses is reference number. [b] Correction was made for hepatic extraction ratio of 0.89 (17) based on ref. 11. [c] An actual value of 57 min was calculated for the rat based on the rat liver perfusion data (see text for detail). [d] These values might be considerably overestimated (see text for detail).

The possibility of a long MTT_h (57.6 min) for doxorubicin in humans (Table I) seems to be substantiated by results of analysis of rat (200–250 g of body weight) liver perfusion data recently reported by Skibba *et al.* (22). The plasma level (μg/ml) *versus* time (min) profile in the reservoir following a bolus dose of 2250 μg of doxorubicin could be approximated by the following biexponential equation, $1.2e^{-0.0711t} + 2.1e^{-0.0041t}$. The estimated V_{ss} based on the standard method (23) was 1003 ml. Since the reservoir had a volume of 150 ml the V_{ss} for the liver should be 853 ml. With that estimated V_{ss} and a Q of 15 ml/min used in their study, one could estimate the MTT_h (based on Eq. 4) to be 57 min.

The true MTT_h of chloroquine in humans might be considerably shorter than estimated here if its distribution between plasma and red blood cells could take place very rapidly, since its concentration in red blood cells has been shown to be much higher (~10 times) than that in plasma (24). The uptake of chloroquine by human red blood cells has been shown to follow saturable kinetics and not to be "instantaneous" (25). Therefore, it appears reasonable to assume that the estimated MTT_h for chloroquine shown in Table I might be either slightly or markedly overestimated. This probably was the case with hydroxychloroquine as well.

The above concepts and findings may be of importance in our study of MAT and the process of GI absorption. They indicate that the conventional method (Eq. 1) may significantly overestimate the true MAT. For digoxin, dactinomycin, doxorubicin, chlorpheniramine, and chloroquine, these overestimations might be 16, 32, 60, 30, and 977 min, respectively (Table I). In theory, the MRT_{po} should be always greater than the MRT_{iv}, even if all the drug is instantaneously absorbed through the GI tract. The degree of differences may vary tremendously with drugs that depends largely on their R_h values (Table I), although the size of the liver and the blood flow may also be important. In this regard it is of interest to point out that in patients with congestive heart failure, hepatic flow might be greatly reduced, and the volume of blood in the liver greatly increased [*i.e.*, up to one extra liter (26)]. Therefore, hepatic transit time for drugs or blood might be much longer than in normal subjects. It is likely that a drug with a larger steady-state volume of distribution in the body would tend to have a longer MTT_h.

It appears that in theory the MTT_h should also be a function of the hepatic extraction ratio, as shown for ethanol (27). For example, when the extraction ratio is unity, no drug molecules will be transported to the general circulation. Therefore, the calculated MTT_h will be zero.

When all drug molecules diffusing out of the hepatic sinusoids are eliminated through biotransformation and/or biliary excretion, the MTT_h for those remaining molecules will essentially equal that for blood. On the other hand, the MTT_h would become the longest for a given drug when the extraction ratio is zero (*i.e.*, no hepatic elimination). Under this condition, transit times for those molecules penetrating hepatic tissues deeply and/or binding strongly hepatic tissues will all be "counted" in the determination of MTT_h for all molecules. Therefore, the data presented in Table I probably represent average values with "normal" hepatic functions.

The MRT analysis has been regarded generally as being model independent. However, it has been shown recently that it might be subject to the influence of the blood-sampling site (28, 29). Although disposition kinetics in the whole body are best represented by systemic arterial data, use of venous data in the MDT analysis should be satisfactory as long as the same site is used for blood sampling throughout the study; this is also true with the MAT analysis shown in Eq. 1 or 2.

In the calculation of MRT_{iv} plasma-level data are most often described by polyexponential equations assuming that the injected drug is instantaneously and homogeneously (kinetically speaking) distributed to the plasma (central) compartment or initial volume of distribution. Such a concept has been questioned recently (29–33). Its potential effect on the determination of MRT_{iv} and MAT seems apparent.

The concept of hepatic first-pass transit times discussed in this communication may also be important in the evaluation of rates of oral absorption using conventional compartmental or deconvolution methods (34–38). This is consistent with an early study (39) which suggested that in the oral absorption rate calculation the calculated rate is based on the same reference sampling point (such as from an arm vein) between intravenous and oral studies. Since one is really only interested in the rate of absorption across the GI membrane, the conventional methods of calculation (34–38) may tend to underestimate the true rate of absorption due to the first "stop" or "trap" in the liver. The extensive trapping in and subsequent slow release from the liver apparently could account for the peculiar peripheral venous plasma level profile of doxorubicin following 30 min of constant intraarterial hepatic infusion to a patient (40). Plasma levels at 10 and 30 min after the beginning of peripheral intravenous infusion of the same dose 3 days later could be estimated (Fig. 1 of ref. 40) to be ~12 and 7 times higher than from the first infusion. The plasma level from intrahepatic infusion started to rise only 60 min after the end of infusion and was 3.3 times higher than from the intravenous infusion 90 min later. Using the 10-min data, the conventional methods would predict the rate of intraarterial infusion or absorption to be only one-twelfth the intravenous infusion. In other words, the infusion or absorption rate could be underestimated by about 92%. This phenomenon might also have occurred in the three dogs receiving portal venous dosing of propranolol in a previous study (41). The infusion rate was repeatedly reduced by ~two-thirds every 7.5 min for 45 min. Systemic arterial propranolol levels were the lowest at the end of the highest rate of infusion (Fig. 5 of ref. 41). One dog peaked at 30 min, another at 60 min, and a third dog plateaued between 20 and 40 min. On the other hand, the highest plasma concentrations were found in the other two dogs studied almost immediately after the same highest rate of infusion (41). The maximum difference at the end of the highest infusion among the five dogs studied could be estimated to be ~150-fold. This dramatic difference was probably primarily a result of the difference in hepatic transit time during the first passage since the plasma levels were quite similar during later periods. Unusually extensive uptake of propranolol by the liver following oral or hepatic portal administration (up to 10 mg/kg) to rats has also been reported (42, 43). The uptake has also been shown (42, 43) to be dependent on the route and dose of administration, thus further complicating the MAT calculation using Eq. 1 or 2.

The concepts of hepatic transit times discussed in this communication also may be useful in the design and evaluation of intrahepatic administration regimens and in the study of hepatic clearance of drugs. The importance of finite hepatic blood transit times and metabolite transfer times in metabolism has been extensively discussed recently (21, 44).

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Errors in Estimating the Unbound Fraction of Drugs Due to the Volume Shift in Equilibrium Dialysis

Keyphrases □ Equilibrium dialysis—volume shift, unbound fraction of drug □ Unbound fraction of drugs—equilibrium dialysis, volume shift

To the Editor:

Equilibrium dialysis is commonly used to estimate serum protein binding of drugs. Consideration of the influence of the volume shift on the unbound fraction has, however, not been addressed until recently (1). The water flux from the buffer side to the serum side during dialysis causes binding protein dilution as well as an overestimation of the unbound fraction. The overestimation is dependent on the extent of the volume shift, the unbound fraction of drugs, and the concentration dependency of binding. Correction for the volume shift is important when the volume shift is substantial and when the unbound fraction of drugs is small.

The molarity of macromolecules in undiluted serum sample is ~1 m*M*, which gives 0.025 atm (263 mm H_2O) of osmotic pressure at 37°. The pressure causes water to migrate from the buffer side to the serum side (1) and expands the dialysis membrane. Because the serum sample is not completely restrained in the dialysis cells and the dialysis membrane, the hydrostatic pressure due to the volume shift is always less than the osmotic pressure. Osmotic equilibrium is actually never reached in this type of equilibrium dialysis. The extent of the volume shift depends on the time used for dialysis. Tozer *et al.* (1) reported an average volume shift of 31% in 16-hr dialysis. Using the same type of dialysis cells and dialysis membrane, we experienced an average volume shift of 10% in 4–6 hr of dialysis. Undue water flux can be avoided by a judicious choice of equilibration time.

Assuming that binding follows the law of mass action, the unbound fraction (f_u) of a drug that has multiple binding sites on a serum binding protein can be expressed as follows:

$$f_u = 1 \Big/ \left[1 + \mathrm{Pt} \sum_{i=1}^{n} 1/(C_u + K\mathrm{d}_i)\right] \qquad \text{(Eq. 1)}$$

where $K\mathrm{d}_i$ is the dissociation constant for binding site i, Pt is the total concentration of binding sites, and C_u is the measured unbound drug concentration. The extent of the volume shift can be defined as the ratio of serum volume before (V_s) and after ($V_{s'}$) dialysis and expressed as:

$$\mathrm{F} = V_s/V_{s'} = \mathrm{Pt}'/\mathrm{Pt} \qquad \text{(Eq. 2)}$$

where Pt′ is the concentration of binding sites after dialysis. In assessing the importance of the volume shift correction, the unbound fractions, with and without water flux correction, need to be compared. Assuming the unbound concentration to be the same with and without a water flux, the unbound fraction without correction for volume shift ($f_{u'}$) is related to the unbound fraction with volume shift correction by:

$$f_{u'} = f_u/[\mathrm{F} \cdot (1 - f_u) + f_u] \qquad \text{(Eq. 3)}$$

or

$$f_u = f_{u'} \cdot \mathrm{F}/(f_{u'} \cdot \mathrm{F} + 1 - f_{u'}) \qquad \text{(Eq. 4)}$$

(See *Appendix* for derivation.) Neglecting the volume shift, the fractional error [$\mathrm{E} = (f_{u'} - f_u)/f_u$] in calculating the unbound fraction is:

$$\mathrm{E} = (1 - \mathrm{F}) \cdot (1 - f_u)/[\mathrm{F} \cdot (1 - f_u) + f_u] \qquad \text{(Eq. 5)}$$

or

$$\mathrm{E} = (1 - \mathrm{F}) \cdot (1 - f_{u'})/\mathrm{F} \qquad \text{(Eq. 6)}$$

It is apparent from Eqs. 5 and 6 that when the volume shift is <10% (F > 0.9), the error introduced in neglecting volume shift is <11%, which is not critical in comparison with other errors in the protein binding determination. However, if the volume shift is >10% and the unbound fraction calculated without the volume shift correction is <0.9, the volume shift should always be considered in calculating the unbound fraction. Equation 4 can be used for the volume shift correction provided that the binding is not concentration dependent in the measured concentration range.

When equilibrium dialysis is used to determine the unbound fraction of a drug with concentration-dependent binding, the transfer of drug from the serum side to the buffer side causes a decrease in the drug concentration on the serum side with a subsequent decrease in the unbound fraction of the drug (1, 2). The complicated correction

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