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Original article

On the mechanism of human intestinal absorption

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Abstract

In order to investigate whether the main step in intestinal absorption in humans is dominated by partition or by diffusion, we have transformed % human intestinal absorption into a first-order rate constant, and have regressed the latter, as log k, against our solvation parameters. The obtained regression coefficients are compared with those for diffusion and partition processes. The coefficients in the log k equation are completely different to those for water/solvent partitions, but are very similar to those for processes (not involving transport through membranes) in which diffusion is the major step. It is suggested that the main step in the absorption process is diffusion through a stagnant mucus layer, together with transfer across the mucus | membrane interface. It is further shown that for strong Bronsted acids and bases, the rate constant for absorption of ionic species is close to that for absorption of the corresponding neutral species, so that to a first approximation the % intestinal absorption can be calculated from properties of the neutral species.

Keywords: Intestinal absorption; Membrane; Partition coefficients; Dissolution

1. Introduction

The mechanism of intestinal absorption has been discussed in numerous books and reviews, [1–4] and a general scheme may be formulated as shown in Fig. 1. If a drug is administered as a solid, then dissolution, k_{diss}, is the first step. The drug then crosses the lumen | gut interface, diffuses across the membrane, exits the membrane, and is then removed by perfusion into the blood stream, k_{perf}. Rowland and Tozer [2] point out that dissolution of a solid may sometimes be the rate-determining step, and Dressman et al. [4] have discussed this in depth. On the other hand, if a drug crosses the intestinal membrane very rapidly, then the perfusion step may be rate controlling. Even if neither the dissolution nor perfusion steps are rate determining, there are still various possible rate-determining steps. Van de Waterbeemd et al. [5] have used the scheme shown in Fig. 1 to explain the transfer of compounds across a water | membrane interface. On their mechanism, the rate constants at each of the membrane interfaces are themselves composite constants, as shown in Fig. 2 for the lumen | membrane interface. A compound has to diffuse through a stagnant layer (SL), on the aqueous side, cross the actual interface, and then diffuse through a SL organic layer on the membrane side. Van de Waterbeemd et al. [5] constructed a set of equations that related to Fig. 2, mostly in terms of the water/membrane partition coefficients, for which water/octanol partition coefficients were used instead. Although all of the analyses of van de Waterbeemd et al. [5,6] were concerned with water/membrane transfers, intestinal absorption was not discussed. Their scheme in Fig. 2 could be considered to be an extension of that in Fig. 1. More recently, Lennernas [7] has suggested that the aqueous SL contributes little to overall intestinal permeability, and that intestinal absorption is

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'membrane controlled'. Lennernas [7] also showed that in vitro measurements of permeability across segments of human intestine were well related to the in vivo estimated time to maximal absorption (though rather poorly to the % absorption) for five drugs. In contrast, Larhed et al. [8] suggest that the mucus layer that covers the surface of the gastrointestinal tract can act as a barrier to drug absorption. Behrens et al. [9] also showed that the mucosal layer on Caco-2 cells was a significant barrier to absorption of testosterone and a number of barbiturates. As regards the effect of solute charge, Larhed et al. [8] showed that the effect of charge on diffusion through the mucus was not very large. Palm et al. [10] found that the un-ionised forms of alfentanil and cimetidine were transported across Caco-2 cells more rapidly than the cationic forms by factors of only 150 and 30, respectively.

To our knowledge, however, there has been no study in which human in vivo data, especially the % absorption, have been generally related (i.e. for a large number of drugs of varied structure) to some mechanistic model of intestinal absorption. In particular, there is a marked lack of comment on the role of strong acids and bases in vivo % absorption. Various workers [11–15] who have related % absorption to drug physicochemical properties do not refer to the problem at all. Yet Rowland and Tozer [2] use gastrointestinal absorption as an example to show that strong acids and bases that are predominantly ionised in the intestinal tract should be transported very slowly, and Sugawara et al. [16] correlate perfusion rates in rat jejunum with various physicochemical descriptors, taking neutral, cationic and anionic compounds separately. Quite recently, Wohnsland and Faller [17] have devised an artificial membrane consisting of an immobilised layer of hexadecane. They determined Pe/pH profiles for the ionisable compounds diclofenac and desipramine. In both cases, significant permeation occurred at pH values where the fraction of neutral species was negligible. Testa and co-workers [18] have determined the partition of a number of ionisable drugs form water to 1,2-dichloroethane. As expected, partition of the ionised forms is much less favoured than partition of the neutral forms. However, Testa and co-workers also showed that neutral species could facilitate transfer of a proton, to an organic phase and hence the neutral species present at the organic side of the interface can become protonated. This might be a very important mechanism of transfer of protonated bases across a polarised interface.

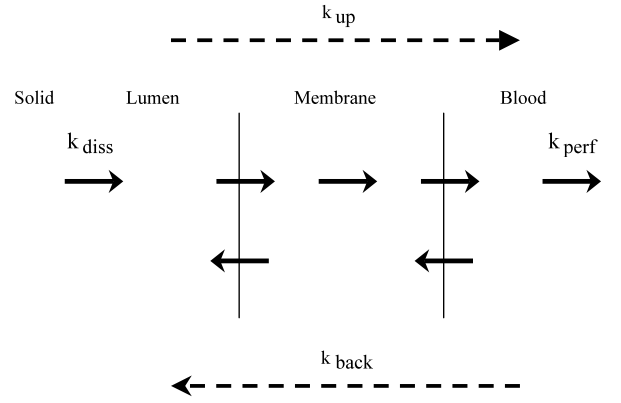

Fig. 1. A general mechanism for intestinal absorption; → and ← represent kinetic paths with a given rate constant.

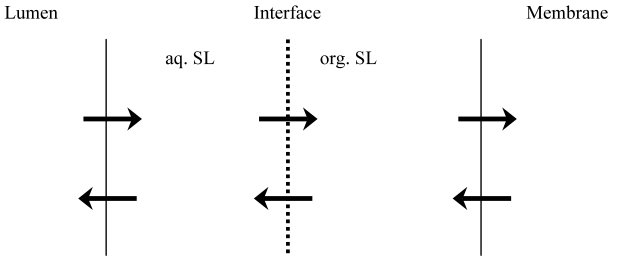

Fig. 2. A mechanism for the path across the lumen | membrane interface; → and ← represent kinetic paths with a given rate constant.

Our aim is to construct a model for passive intestinal absorption, and to relate this to our large data base [19] of in vivo % absorption. Excluded from our analysis are compounds where dissolution of the solid could be the rate-determining step. Any effect of first pass metabolism is also excluded by the way that the absorption data is obtained. Hence the in vivo data discussed here are absorption and not bioavailability data.

2. Chemistry

2.1. General analysis

We start with our general solvation equation, Eq. (1), that we have used [19–21] to correlate and to interpret a wide variety of passive transport properties.

$$SP = c + e\mathbf{E} + s\mathbf{S} + a\mathbf{A} + b\mathbf{B} + v\mathbf{V} \quad (1)$$

The dependent variable in Eq. (1) is a property of a series of solutes in a given system. In this work, SP can be the % intestinal absorption, or can be some physicochemical property such as $\log P$ where P is a given water/solvent partition. The independent variables in Eq. (1) are solute descriptors as follows: [20,21] we use a simplified terminology, and give the original symbols in parentheses. **E** (R_2) is the solute excess molar refractivity in units of M/10, S (π_2^H) is the solute dipolarity/polarisability, **A** ($\Sigma \alpha_2^H$) and **B** ($\Sigma \beta_2^H$) are the overall or summation hydrogen bond acidity and basicity, and **V** is the McGowan characteristic volume [22] in units of (M)/100.

We have applied Eq. (1) to human % absorption for drugs, %Abs leading to Eq. (2) where n is the number of data points (drugs), r is the correlation coefficient, SD is the standard deviation and F is the F-statistic [19]; the SD values for the coefficients are given below the coefficients themselves. Unlike many previous workers, we analysed the influence of strong Bronsted acids and bases, but, by inspection, could find very little effect. Thus Eq. (2) includes Bronsted acids and bases, with no correction for ionisation at all.

$$\%\mathrm{Abs} = \underset{3.8}{92.0} + \underset{2.75}{2.94}\mathbf{E} + \underset{2.43}{4.10}\mathbf{S} - \underset{2.66}{21.70}\mathbf{A} - \underset{1.68}{21.10}\mathbf{B} + \underset{2.08}{10.60}\mathbf{V} \quad (2)$$

$$n = 169,\ r^2 = 0.74,\ \mathrm{SD} = 14\%,\ F = 93$$

$$\%\mathrm{Abs} = \underset{4.2}{94.0} + \underset{2.74}{2.90}\mathbf{E} + \underset{2.70}{2.71}\mathbf{S} - \underset{2.79}{20.70}\mathbf{A} - \underset{1.68}{20.90}\mathbf{B} + \underset{2.14}{11.20}\mathbf{V} - \underset{2.63}{3.41}\mathbf{I} \quad (3)$$

$$n = 169,\ r^2 = 0.74,\ \mathrm{SD} = 14\%,\ F = 78.$$

In an attempt to define any influence of strong Bronsted acids and bases more rigorously, we incorporated an indicator variable for acids with $pK_a < 4.5$ and bases with $pK_a > 8.5$. The resulting equation, Eq. (3), is very close to Eq. (2). The indicator variable term contributes to the % absorption only very slightly, in the sense that strong Bronsted acids and bases are absorbed only 3% less than calculated from properties of their neutral forms.

Eq. (2) is an empirical equation in the sense that the 'quality' of the dependent variable is not compatible with the independent variables, that are free-energy related. In order to deal with questions about mechanism, etc. we therefore have to transform the % absorption into a free-energy related quantity, of which $\log k$, where k is the overall rate constant for absorption, is the most appropriate. Now in intestinal absorption, the drug concentration on the receiving site (portal vein) is usually negligible in relation to that on the driving site (intestinal tract), where there a quantity of the substance dissolved in the small intestinal fluid. In addition we deal only with a mechanism that does not involve rate-determining dissolution of a solid, see Fig. 1. Then, the absorption rate-determining step is passive diffusion across the membrane system. Within these reasonable simplifications, the rate of diffusion follows first-order kinetics, with an overall rate constant, k, given by Eq. (4) where C_w is the drug concentration in the intestinal tract at a time t. Integration of Eq. (4) leads to Eq. (5), where C_w^0 is the drug concentration at $t = 0$. If we take C_w^0 as 100% (that is the initial concentration) and C_w as the observed % absorption at some given time, t, then Eqs. (6) and (7) follow.

$$dC_w/dt = -kC_w \quad (4)$$

$$\ln(C_w^0 - C_0)/C_w^0 = -kt \quad (5)$$

$$\ln[1 - (\%/100)] = -kt \quad (6)$$

$$\log k = \log\{\ln[100/(100 - \%)]\} - \log t \quad (7)$$

Now Eqs. (6) and (7) collapse when the % absorption is 0 or 100, and so can only be applied to drugs that are absorbed neither too rapidly nor too slowly. Under these conditions, we may make the assumption that the time to maximum absorption, and hence the $\log t$ term in Eq. (7), is constant from one drug to another. This does not conflict with results summarised by Lennernas, [7] who showed that for drugs with 100% absorption, the time to maximum absorption was shorter than for drugs with smaller % absorption. With the above assumption, Eq. (8) follows; the constant $\log t$ term is now subsumed into the equation c-constant.

$$\log\{\ln[100/(100 - \%)]\} = c + e\mathbf{E} + s\mathbf{S} + a\mathbf{A} + b\mathbf{B} + v\mathbf{V} \quad (8)$$

3. Results and discussion

From our data set on human intestinal absorption, [19] we have 127 compounds that have a % absorption other than 0 or 100. Note that the 127 data set does not include zwitterionic compounds, nor very hydrophilic compounds such as urea or glucose. Details are in Table 1, and application of Eq. (8) leads to Eq. (9)

$$\log\{\ln[100/(100 - \%\mathrm{Abs.})]\} = \underset{0.092}{0.544} - \underset{0.075}{0.025}\mathbf{E} + \underset{0.062}{0.141}\mathbf{S} - \underset{0.068}{0.409}\mathbf{A} - \underset{0.042}{0.514}\mathbf{B} + \underset{0.059}{0.204}\mathbf{V} \quad (9)$$

$$n = 127,\ r^2 = 0.80,\ \mathrm{SD} = 0.29,\ F = 94$$

The constants in Eq. (9) bear no relation at all to those in numerous equations we have constructed for water/solvent partition equilibria, as $\log P$, or for rates of transfer from water to another phase as $\log k$. This can be seen from Table 2, that includes some representative water/solvent partitions [23,24] as well as partition from water to plant matrix, [25] and to a C_{18} Empore disk [26]. The rate processes in Table 2 are for skin permeation [27] and plant cell permeation [28] both from water.

The detailed analysis of van de Waterbeemd et al. [5] on the water/octanol and water/dibutyl ether systems helps to provide an explanation through the models shown in Figs. 1 and 2. These workers measured the water/octanol and water/dibutylether partition, P, of a series of sulfonamides, and also the rate of uptake of the sulfonamides from water to the organic solvents, k_{up}. From P and k_{up} they were able to calculate the rate of off-loading of the solutes from the organic solvent back to water through the equation,

$$P = k_{up}/k_{back} \quad (10)$$

Table 1
The 127 compounds used in Eq. (9)

Name	%Abs	Log k	Name	%Abs	Log k
Lactulose	0.6	−2.22	Oxyfedrine	85	0.28
Kanamycin	1	−2.00	Tolbutamide	85	0.28
Neomycin	1	−2.00	Topiramate	86	0.29
Streptomycin	1	−2.00	Bupropion	87	0.31
Ouabain	1.4	−1.85	Lamivudine	87	0.31
Acarbose	2	−1.69	Pindolol	87	0.31
Cidofovir	3	−1.52	Felodipine	88	0.33
Ganciclovir	3	−1.52	Moxonidine	88	0.33
Adefovir	16	−0.76	Nitrendipine	88	0.33
k-Strophanthoside	16	−0.76	Saccharin	88	0.33
Mannitol	16	−0.76	Dihydrocodeine	89	0.34
Foscarnet	17	−0.73	Oxazepam	89	0.34
Lincomycin	28	−0.48	Sultopride	89	0.34
Netivudine	28	−0.48	Tenidap	89	0.34
Fosmidomycin	30	−0.45	Alprazolam	90	0.36
Fosfomycin	31	−0.43	Amphetamine	90	0.36
Ascorbic acid	35	−0.37	Betaxolol	90	0.36
Famotidine	38	−0.32	Chloramphenicol	90	0.36
Metaproterenol	44	−0.24	Felbamate	90	0.36
Sulpiride	44	−0.24	Ketorolac	90	0.36
Cymarin	47	−0.20	Meloxicam	90	0.36
Rimiterol	48	−0.18	Nisoldipine	90	0.36
Amiloride	50	−0.16	Nizatidine	90	0.36
Atenolol	50	−0.16	Phenytoin	90	0.36
Guanoxon	50	−0.16	Sulindac	90	0.36
Metformin	53	−0.12	Terazosin	90	0.36
Nadolol	57	−0.07	Tramadol	90	0.36
Sumatriptan	57	−0.07	Hydrocortisone	91	0.38
Fenoterol	60	−0.04	Naloxone	91	0.38
Pirbuterol	60	−0.04	Isradepine	92	0.40
Reproterol	60	−0.04	Ketoprofen	92	0.40
Ziprasidone	60	−0.04	Alprenolol	93	0.42
Furosemide	61	−0.03	Amrinone	93	0.42
Terbutaline	62	−0.01	Codeine	95	0.48
Cimetidine	64	0.01	Fluconazole	95	0.48
Metolazone	64	0.01	Flumazenil	95	0.48
Hydrochlorothiazide	65	0.02	Ibuprofen	95	0.48
Recainam	71	0.09	Labetalol	95	0.48
Cycloserine	73	0.12	Metoprolol	95	0.48
Propylthiouracil	76	0.15	Oxprenolol	95	0.48
Famciclovir	77	0.17	Practolol	95	0.48
Mercaptoethane	77	0.17	Scopolamine	95	0.48
Urapidil	78	0.18	Sotalol	95	0.48
Acebutolol	80	0.21	Timolol	95	0.48
Acetaminophen	80	0.21	Bumetanide	96	0.51
Dexamethasone	80	0.21	Torasemide	96	0.51
Ethambutol	80	0.21	Trapidil	96	0.51
Guanabenz	80	0.21	Antipyrine	97	0.54
Isoniazid	80	0.21	Clofibrate	97	0.54
Methadone	80	0.21	Disulfiram	97	0.54
Omeprazole	80	0.21	Trimethoprim	97	0.54
Digoxin	81	0.22	Venlafaxine	97	0.54
Flecainide	81	0.22	Atropine	98	0.59
Piroximone	81	0.22	Lamotrigine	98	0.59
Quinidine	81	0.22	Minoxidil	98	0.59
Methylprednisolone	82	0.23	Tolmesoxide	98	0.59
Mifobate	82	0.23	Viloxazine	98	0.59
Sorivudine	82	0.23	Warfarin	98	0.59
Acetylsalicylicacid	84	0.26	Carfecillin	99	0.66
Bromazepam	84	0.26	Naproxen	99	0.66
Captopril	84	0.26	Nordiazepam	99	0.66
Propiverine	84	0.26	Prednisolone	99	0.66
Lansoprazole	85	0.28	Propranolol	99	0.66
Morphine	85	0.28			

Table 2
System coefficients for water/phase transfers

Phase	SP	*e*	*s*	*a*	*b*	*v*
Intestinal absorption	$\log k$	−0.02	0.14	−0.41	−0.51	0.20
Water–octanol	$\log K(P)$	0.56	−1.05	0.03	−3.46	3.81
Water–cyclohexane	$\log K(P)$	0.78	−1.68	−3.74	−4.93	4.58
Water–hexadecane	$\log K(P)$	–	–	–	–	–
Water–chloroform	$\log K(P)$	0.16	−0.39	−3.19	−3.44	4.19
Water–plant matrix	$\log K$	0.60	−0.41	−0.51	−4.10	3.91
Water–C_{18} disk	$\log K(P)$	0.35	−0.07	−0.63	−1.96	2.85
Water–human skin	$\log k_{up}$	0.44	−0.49	−1.48	−3.44	1.94
Water–plant cell	$\log k_{up}$	0.00	−0.87	−3.14	−1.66	0.73

Note that k_{up} and k_{back} are the overall rate constants, see Fig. 1. It was found [5] that a plot of $\log k_{up}$ or $\log k_{back}$ against $\log P$ consisted of three sections, as shown in Fig. 3. Considering just k_{up}, the first section shows $\log k_{up}$ increasing linearly with $\log P$, the second section is an intermediate one leading into the third section where $\log k_{up}$ is essentially constant, and hence independent of $\log P$. Van de Waterbeemd et al. [5] interpreted the plot of $\log k_{up}$ against $\log P$ as follows. In the first section, the factors that influence $\log k_{up}$ are similar to those that influence $\log P$, but as k_{up} becomes larger and larger it reaches a diffusion controlled rate limit in section three. This rate-limiting step is due to diffusion through the SL, between the two phases. A similar rate-limiting step takes place as k_{back} becomes larger and larger (now at small values of $\log P$). Leahy et al. [29] have confirmed these findings through a study of a diverse range of solutes in the water/octanol, chloroform, isooctane and propylene glycol dipelargonate systems, and were able to construct plots that corresponded to sections 1 and 2 in Fig. 3. In the systems of van de Waterbeemd et al. [5] and Leahy et al. [29] no membrane is involved at all, so that there is no question of any 'membrane control', cf. Ref. [7], and it is reasonable to ascribe the diffusion limit to k_{up}, to the effect of the aqueous SL.

A rather different type of system in which the organic phase is a C_{18} Empore™ extraction disc consisting of 90% w/w octadecyl-silica sorbent embedded in 10% w/w PTFE microfibrils has been examined by Abraham and Green [26]. Values of k_{up} from water were obtained for a series of diverse solutes. There is a very small dependence on solute structure, see Eq. (11) where the numerical values of the coefficients are small,

$$\log k_{up} = \underset{0.11}{-5.34} + \underset{0.09}{0.08}\mathbf{E} + \underset{0.11}{0.20}\mathbf{S} - \underset{0.09}{0.08}\mathbf{A} - \underset{0.07}{0.28}\mathbf{B} + \underset{0.11}{0.33}\mathbf{V} \quad (11)$$

$$n = 21,\ r^2 = 0.95,\ \mathrm{SD} = 0.08,\ F = 30$$

Even for strict diffusion, in a homogeneous medium, there may still be a small dependence on solute properties. Data are available for the diffusion of organic compounds in water [30,31] at 298 K, with diffusion coefficients, Dc, in 10^5 cm^2 s^{-1}. The usual way of analysing Dc is through the function 1/Dc, and we find the equation,

$$1/\mathrm{Dc} = \underset{0.052}{0.434} - \underset{0.082}{0.157}\mathbf{E} - \underset{0.072}{0.138}\mathbf{S} + \underset{0.046}{0.114}\mathbf{A} + \underset{0.056}{0.126}\mathbf{B} + \underset{0.088}{0.868}\mathbf{V} \quad (12)$$

$$n = 49,\ r^2 = 0.752,\ \mathrm{SD} = 0.107,\ F = 26$$

However, in order to compare diffusion coefficients with the absorption function $\log k$, we need to correlate log Dc values,

$$\log \mathrm{Dc} = \underset{0.024}{0.268} + \underset{0.037}{0.054}\mathbf{E} + \underset{0.032}{0.073}\mathbf{S} - \underset{0.021}{0.062}\mathbf{A} - \underset{0.025}{0.041}\mathbf{B} - \underset{0.040}{0.399}\mathbf{V} \quad (13)$$

$$n = 49,\ r^2 = 0.754,\ \mathrm{SD} = 0.048,\ F = 26$$

The units of Dc are 10^5 cm^2 s^{-1} but change to any other units would affect only the constant term in Eq. (13) and would leave the coefficients unchanged. As expected for diffusion in a homogeneous medium, 1/Dc is proportional to the size of the solute, and log Dc is then related to a negative term in *V*. However, a

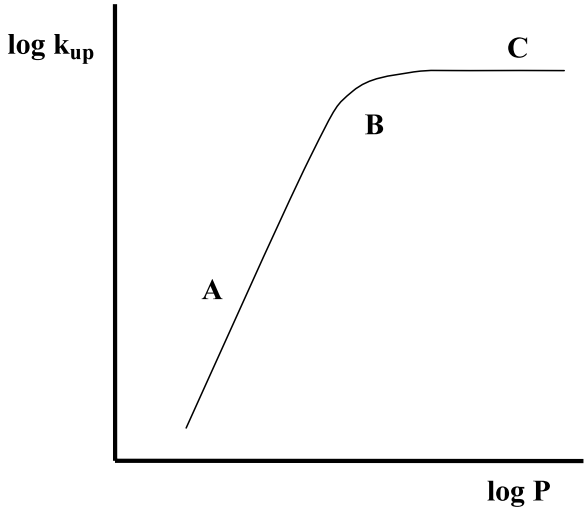

Fig. 3. The scheme of van de Waterbeemd et al. [5] for partition and uptake from water to solvents, showing how $\log k_{up}$ varies with $\log P$.

Table 3
System coefficients for diffusion processes

Phase	SP	*e*	*s*	*a*	*b*	*v*
Intestinal absorption, Eq. (9)	log *k*	0.02	0.14	−0.41	−0.51	0.20
C_{18} disk, Eq. (12)	log k_{up}	0.08	0.20	−0.08	−0.28	0.33
Ethanol diffusion, Eq. (15)	log Dc [a]	0.01	−0.04	−0.38	−0.05	−0.23
Water diffusion, Eq. (13)	log Dc [a]	0.05	0.07	−0.06	−0.04	−0.40
Lateral diffusion, Eq. (16)	log *D*/*h*	0.58	−0.39	−0.05	−0.44	0.35

[a] Dc is the limiting diffusion coefficient in bulk ethanol or in bulk water solvent.

surprising feature of Eq. (13) is the very small numerical size of the coefficients. It may be argued that the data we have used has been assembled from results of several workers using different experimental techniques and so is not very soundly based. Fortunately, Chen and Chan [32] have determined limiting diffusion coefficients of 27 aromatic compounds in ethanol, and we find the equations,

$$1/\mathrm{Dc} = \underset{0.037}{0.197} + \underset{0.033}{0.022}\mathbf{E} - \underset{0.049}{0.007}\mathbf{S} + \underset{0.027}{0.853}\mathbf{A} + \underset{0.055}{0.105}\mathbf{B} + \underset{0.047}{0.468}\mathbf{V} \quad (14)$$

$$n = 27,\ r^2 = 0.989,\ \mathrm{SD} = 0.027,\ F = 385$$

$$\log \mathrm{Dc} = \underset{0.025}{0.412} + \underset{0.022}{0.008}\mathbf{E} - \underset{0.033}{0.037}\mathbf{S} - \underset{0.018}{0.388}\mathbf{A} - \underset{0.037}{0.051}\mathbf{B} - \underset{0.031}{0.226}\mathbf{V} \quad (15)$$

$$n = 27,\ r^2 = 0.977,\ \mathrm{SD} = 0.018,\ F = 186$$

Eqs. (14) and (15) are very comparable to Eqs. (12) and (13); both sets show that even for limiting diffusion in homogeneous media, there are effects due to hydrogen bonding.

Although van de Waterbeemd et al. [5] gave numerical values of k_{up} for all three sections of Fig. 3, there are not enough solutes in the separate sections A and C to carry out an analysis through Eq. (1). However, we can still compare the coefficients in Eq. (9) with those for which diffusion seems to be the major process, as shown in Table 3. The coefficients for the processes in Table 2 are all far away from the coefficients for the processes listed in Table 2. By comparison to the processes in Table 3, there is a remarkable similarity between the three diffusion equations in Table 3. Since neither Eq. (13) nor Eq. (15) refer to diffusion through a membrane, it is not necessary to postulate that such a process is part of the rate-limiting step. In this context, the work of Johnson et al. [33], Mitragotri et al. [34] and Pugh et al. [35] is extremely important. Johnson et al. [33] examined permeation through the skin *stratum corneum* and concluded that lateral diffusion in the membrane bilayer was the primary transport step. On the other hand, Mitragotri et al. [34] have argued that in general it is the actual interfacial transport at the membrane boundary that is the rate-determining step. The two comments are not incompatible. In skin permeation a solute has to diffuse across about 100 bilayers; the rate-determining step then becomes lateral diffusion along a bilayer, as the solute seeks to find a hole through which to pass to the next bilayer.

Pugh et al. [35] calculated lateral diffusion in the *stratum corneum* as $\log D/h = \log \mathrm{kp} - \log \mathrm{Ksc}$ where kp is the rate constant for permeation from water, and Ksc is the water–skin partition coefficient. Abraham and Martins [27] calculated values of $\log D/h$ in the same way, and obtained Eq. (16) after regression against our usual parameters.

$$\log D/h = \underset{0.168}{-6.324} - \underset{0.267}{0.579}\mathbf{E} - \underset{0.201}{0.391}\mathbf{S} - \underset{0.261}{0.048}\mathbf{A} - \underset{0.288}{0.440}\mathbf{B} + \underset{0.194}{0.354}\mathbf{V} \quad (16)$$

$$n = 37,\ r^2 = 0.864,\ \mathrm{SD} = 0.377,\ F = 39$$

The equation for lateral diffusion is much nearer to Eq. (9) than is the equation for skin permeation, see Table 3.

Our conclusion is that the available evidence suggests diffusion in the stagnant mucose layer together with the actual interfacial (mucus | membrane) transfer as the rate-determining step in human intestinal absorption, and we compare Eq. (9) with other equations in a quantitative manner later.

3.1. Bronsted acids and bases

We now seek to understand the behaviour of strong Bronsted acids and bases in human intestinal absorption. Our original analysis suggested that the effect of these ionisable compounds on the observed % absorption was very small—of the order of only a reduction of about 3% for acids of $pK_a < 4.5$ and bases of $pK_a > 8.5$. Now since the pH of the upper small intestine is around 6–7, both of these types of compound will be mainly in an ionised form. How are such ionised species absorbed?

One possibility is that the ionic⇔neutral equilibrium, Eq. (17), provides a pathway for the ionic species to be absorbed in an indirect way, through the mechanism shown in Eq. (18); I and N refer to the ionic and neutral species, and k^N to the second (rate-determining) step. Note that all our equations and discussion on the equilibrium, Eq. (17) refer to monoprotic species.

$$I + H^+ \Leftrightarrow N \quad K_a = [H^+][I]/[N] \tag{17}$$

$$I \Leftrightarrow N \xrightarrow{k^N} \text{Absorption} \tag{18}$$

The rate of absorption is given by

$$r = k^N\, N \tag{19}$$

and if we combine Eq. (17) with Eq. (19) we obtain Eq. (20), where H denotes $[H^+]$, and T denotes the formal solute concentration, $[I] + [N]$,

$$r = k^N\, T/(1 + K_a/H) \tag{20}$$

The observed rate constant is then given by,

$$k^{obs} = k^N/(1 + K_a/H) \tag{21}$$

Now suppose we take $H = 10^{-6.5}$, we can then calculate the expression for k^{obs} if we have a series of acids with various values of K_a, as shown in Table 4. Acids and bases that are ionised will appear to permeate by orders of magnitude less than the neutral species. This would greatly impact on the % absorption, and we can conclude that the mechanism shown in Eq. (18) does not operate in any major way, as far as we can tell from the data on % absorption in man.

Now if we assume that both the ionic species, I, and the neutral species, N, are directly absorbed, the observed rate constant will be given by,

$$k^{obs}\, T = k^I\, I + k^N\, N \tag{22}$$

and from Eqs. (17) and (22) we have,

$$k^{obs}\, T = N[k^I\, K_a/H + k^N] \tag{23}$$

Now the total substrate, T, is given by,

$$T = N + I = N + K_a\, N/H \tag{24}$$

Table 4
Analysis of absorption of Bronsted acids on Eq. (21), with $H = 10^{-6.5}$

K_a	Expression for k^{obs}
10^{-9}	$0.99\, k^N$
10^{-7}	$0.76\, k^N$
10^{-5}	$0.03\, k^N$
10^{-3}	$0.0003\, k^N$

Table 5
Analysis of the absorption of Bronsted acids on the mechanism of Eq. (25) with $H = 10^{-6.5}$

K_a	Expression for k^{obs}	Expression for k^{obs} when		
		$k^I/k^N = 0.5$	$k^I/k^N = 0.01$	$k^I/k^N = 0.001$
10^{-9}	$(10^{-2.5}\, k^I + k^N)$	$1.00\, k^N$	$1.00\, k^N$	$1.00\, k^N$
$10^{-6.5}$	$(0.5k^I + 0.5k^N)$	$0.75\, k^N$	$0.51\, k^N$	$0.50\, k^N$
10^{-5}	$(0.97k^I + 0.031k^N)$	$0.52\, k^N$	$0.04\, k^N$	$0.03\, k^N$
10^{-3}	$(k^I + 10^{-3.5}\, k^N)$	$0.50\, k^N$	$0.01\, k^N$	$0.001\, k^N$

and if we combine Eqs. (23) and (24), we find that k^{obs} depends on H and K_a

$$k^{obs} = [(k^I\, K_a + k^N\, H]/(H + K_a) \tag{25}$$

Now again, consider a constant pH so that $H = 10^{-6.5}$. We can then calculate what expression we should get for substrates of various K_a values, as shown in Table 5.

It is clear that if the ratio k^I/k^N becomes very small (i.e. the ionic species is very poorly absorbed), then the observed rate constant will decrease enormously for species that are strong Bronsted acids or bases. This is not the case for human intestinal absorption. On the other hand, as k^I/k^N approaches unity, so the effect of the ionic species on the observed rate constant becomes smaller and smaller. We can calculate from Eq. (7) that if k^I/k^N is 0.86, then strong Bronsted acids and bases will reduce the observed % absorption by 5%, and even if k^I/k^N is 0.72 the observed absorption is reduced by only 10%. Our analysis using an indicator variable, Eq. (3), suggests a reduction of only 3%, so with an allowance for some considerable experimental error, we can conclude that in general k^I/k^N cannot be less than about 0.8–0.7 in human intestinal absorption.

Morishita et al. [36] have studied the intestinal absorption of a number of acidic sulfonamides in the rat through an in situ perfusion method that allowed the pH to be adjusted. Under these conditions, it was possible to determine rate constants for absorption of the neutral and the anionic forms. Values of k^I/k^N ranged from 1.0 to 0.46 so that in this, admittedly different, system ionic species are absorbed almost as readily as the neutral species. Interestingly, the effect of compound structure on k^N was quite small. Values of $\log k^N$ cover only 1.06 units, as compared to 3.15 log units for the variation in $\log P_{oct}$, so that this absorption may also be diffusion controlled.

Our calculations on ionic absorption are thus compatible with the findings of Morishita et al. [36] and also with the observation of Larhed et al. [8] that the effect of charge on diffusion through 1.5% pig intestinal mucus was not very large. The observations of Palm et al. [10] on permeation through Caco-2 cells show a rather larger (retarding) effect of ionic charge, but the Caco-2 system is not the same as the intestinal systems of Morishita et al. [36] and of Larhed et al. [8]. It is worth noting that although we, and Morishita et al. [36], have suggested that ionic species can be absorbed directly, the actual mechanism of ionic absorption remains to be elucidated. It is known that α-aminoacids and ionic species diffuse readily in aqueous solution, [30] and so some other barrier than diffusion in the SL must account for the varying absorption of zwitterions and ionic species. It is possible that diffusion across the actual SL/membrane boundary is a barrier to certain zwitterions, but that charged species might diffuse

Table 6
Characterisation of systems

Phase	No.	SP	θ	δ-CO	δ-PC	δ-NL
Intestinal absorption, Eq. (9)	1	$\log k$	0	0	0	0
Water–octanol	2	$\log K(P)$	50	6.0	6.1	6.5
Water–cyclohexane	3	$\log K(P)$	31	10.0	10.0	10.0
Water–hexadecane	4	$\log K(P)$	31	9.6	9.5	9.7
Water–chloroform	5	$\log K(P)$	29	7.1	5.8	7.6
Water–plant matrix	6	$\log K$	41	5.8	6.1	7.1
Water–C_{18} disk	7	$\log K(P)$	43	3.3	3.4	4.0
Water–human skin	8	$\log k_{up}$	26	4.6	4.6	4.8
Water–plant cell	9	$\log k_{up}$	34	3.7	4.7	4.3
Lateral diffusion, Eq. (16)	10	$\log D/h$	64	1.1	1.7	1.0
Water–C_{18} disk, Eq. (12)	11	$\log k_{up}$	38	0.6	0.7	0.5
Diffusion in ethanol, Eq. (15)	12	log Dc	65	0.8	0.3	0.9
Diffusion in water, Eq. (13)	13	log Dc	95	1.1	0.5	1.2

across as ion-pairs. Certainly, ion-pair formation with cations derived from typical drug bases will be much more favoured than ion-pair formation with tetraalkylammonium cations. Compare R_3N–$H^+\cdots X^-$ with $R_4N^+\cdots X^-$. In general, it might be expected that membrane transport would be more likely for charged compounds where the charge is delocalised, than for charged species where the charge is localised on one or two atoms.

Yoshida and Topliss [37] have constructed a QSAR for classification of human oral bioavailability. In contrast to our findings, and those of Morishita et al. [36] on intestinal absorption, they find that Bronsted acids have a better bioavailability than neutral species, and that Bronsted bases have lower bioavailability than neutral species. It is worth pointing out that intestinal absorption is not the same as bioavailability. There are many factors involved in the latter, of which intestinal absorption is only one.

3.2. Characterisation of the absorption system

We have suggested that our LFER equation for $\log k$ for intestinal absorption, Eq. (9), is rather similar to those for the rate of uptake from water onto a C18 disk, and for diffusion in water and in ethanol, as well as to that for lateral diffusion in the *stratum corneum*. It would be useful if we had a rigorous method for the comparison of these LFERs, and, indeed, for the characterisation of LFERs in general. Ishihama and Asakawa [38] have put forward a very elegant method for the comparison of LFERs, say SP1 and SP2, as regards correlation. They define an angle, θ, between the vectors of the coefficients in SP1 and SP2 such that if the dependent variable in SP1 is well correlated with that in SP2 then θ is near to zero. However, if the two dependent variables are not well correlated then θ deviates from zero. Since θ can be calculated from the coefficients in SP1 and SP2, the method is extremely convenient. We give in Table 6, values of θ obtained by the method of Ishihama and Asakawa [38], taking $\log k$ for intestinal absorption as the standard system. None of the other processes listed in Table 6 has θ anywhere near to zero, and so we conclude that as regards correlation, none of them will be very good models for intestinal absorption. This has considerable implications as regards predictive algorithms based on water/octanol partition, as $\log P_{oct}$. We find that θ is quite large, at 50, and so $\log P_{oct}$, will be a very poor linear correlative predictor of $\log k$. We have found also that θ is far from zero for %Abs and $\log P_{oct}$. Indeed, Palm et al. [9] showed that there was almost no correlation between %Abs and $C\log P$ for 20 drugs. However, we stress that the data set of compounds that we have used for %Abs and $\log P_{oct}$ are not the same, and so for some data sets there may be linear correlation between these parameters.

Although the θ-parameter is useful as a linear correlative comparison, it is not very useful as regards any chemical comparison of LFERs. Suppose the coefficients in SP2 were all 1/10 of those in SP1, the two systems would be regarded chemically as quite different, and yet θ would still be zero. A very simple way of comparing coefficients in two LFERs is to sum the absolute differences between the coefficients, c_i, in the two LFERs as shown in Eq. (26). The factor 1/5 is introduced so as to give the average difference in the five coefficients e to v. Of course, such a simple method will only work if the dependent variables in SP1 and SP2 refer to the same type of quantity (in this work either the logarithm of a rate constant or the logarithm of an equilibrium constant), and if the various descriptors cover about the same range of values.

$$\delta\text{-CO} = (\sum|c_1 - c_2|)/5c = e \text{ to } v \quad (26)$$

The obtained values of δ-CO are given in Table 7. The three examples of water/solvent partition, Nos. 2–4, are

Table 7
Rank order of difference from log k for intestinal absorption, system No. 1, of the systems listed in Table 4

δ	PCA	NL	θ
1	1	1	1
11	12	11	8
12	13	12	5
10	11	10	3
13	10	13	4
7	7	7	9
9	8	9	11
8	9	8	6
6	5	2	7
2	2	6	2
5	6	5	10
4	4	4	12
3	3	3	13

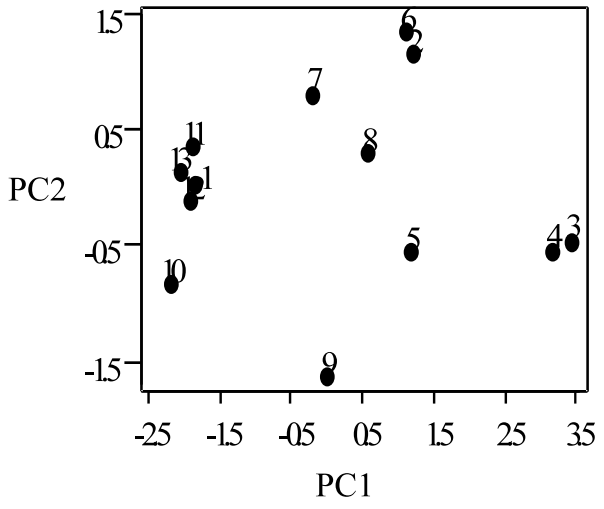

Fig. 4. A plot of PC2 vs. PC1 for the coefficients in Table 6; numbers refer to systems in Table 6.

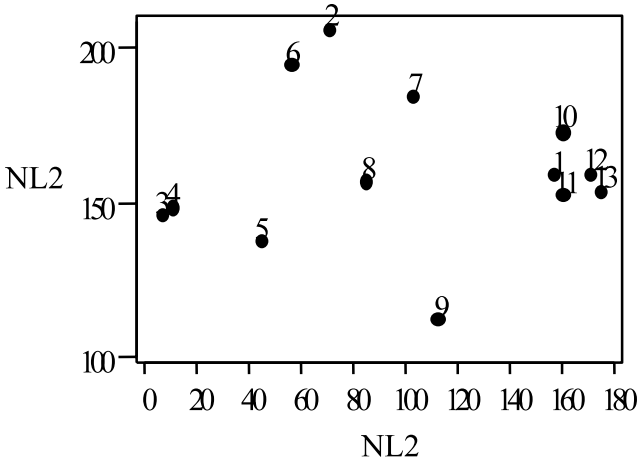

Fig. 5. A non-linear map of the coefficients in Table 6; numbers refer to systems in Table 6.

all chemically far away from intestinal absorption, but the four diffusion controlled processes (Nos. 10–13) are all close to intestinal absorption, in that the chemical factors that influence the processes will be quantitatively very similar.

The use of principal components analysis (PCA) is another method that can be used to compare LFERs. We have carried out a PCA on the coefficients of the systems shown in Table 6, and find that the first two PCs account for 88% of the information. A plot of the scores of PC2 versus PC1 from the correlation matrix, see Fig. 4, shows that processes Nos. 10–13 are very close to intestinal absorption, and that the three water/solvent partitions, Nos. 2–4 are far away. This can be put on a quantitative basis by calculating the geometric distance, δ-PC, of the points in Fig. 4 from the point for intestinal absorption (no 1). The actual numerical values of δ-PC are arbitrary, and it is the relative values that are significant.

We can also show graphically the relationship between the 13 systems in Table 3 through the technique of non-linear mapping (NLM) [39,40]. In Fig. 5 is a two-dimensional non-linear map in which the similarity between the LFERs is given by the distance between the corresponding points. It is again quite clear that processes Nos. 10–13 are very close to intestinal absorption, and that the three water/solvent partitions, Nos. 2–4, are far away. The geometric distances between the points on the NL map, δ-NL, are shown in Table 6. Again, the absolute values are arbitrary, and in Table 6 we have put them on a scale so that the distance between the standard process (no 1) and water–cyclohexane (no 3) is equal to 10.

The simple Eq. (26), PCA, and NLM, all lead to essentially the same results, with almost the same rank order of difference from intestinal absorption, see Table 7. However, the similarity between results from PCA and NLM must be due in part to the fact that the first two PCs account for 88% of the information. If the information content of the remaining PCs becomes more significant, then we would not expect PCA and NLM to yield similar results. The three above methods refer to 'chemical' differences, that is to quantitative differences in the chemical factors that influence transport systems. The rank order of difference from log k for intestinal absorption as obtained from the θ-parameter is not at all similar to those from the other three methods, see Table 7. As mentioned above, this is because the θ-method refers to linear correlative differences, whilst the other methods we have used refer to chemical differences.

The θ-method of Ishihama and Asakawa [38] suggests that human intestinal absorption will not be well correlated in a linear equation by log P_{oct}. The simple Eq. (26), and the more complicated analyses through PCA and NLM, all lead to the conclusion that the factors that influence human intestinal absorption are

quantitatively not the same as those that influence water/solvent partitions and a number of other processes in which solutes are transferred from water to other phases. The factors are similar, however, to those that influence diffusion controlled processes, viz. (1) the rate of diffusion of solutes in water; (2) the rate of diffusion in ethanol solution; (3) the rate of uptake of solutes onto a C_{18} disk; and (4) lateral diffusion in the *stratum corneum*. However, it is difficult to suggest the latter as a realistic model for intestinal absorption, because of the unique layer-like structure of the *stratum corneum*. On the other hand, a mechanism involving rate-determining diffusion through a stagnant mucosal layer is compatible with the suggestions of Larhed et al. [8] and Behrens et al. [9] and with the experimental results of Wohnsland and Faller [17] on water–hexadecane permeation. It also accounts for the very small effect of ionisable species, because ions are known to diffuse quite rapidly in water [30]. We suggest that the comparison of the LFER for $\log k$ for intestinal absorption with LFERs for the various processes listed in Table 6 is a valuable probe of mechanism, and illustrates again the interpretive power of our general solvation equation.

Although our equation for $\log k$ is chemically very close to those for diffusion in a homogeneous medium, it includes a small positive v-coefficient, instead of the small negative v-coefficients in Eqs. (13) and (15). Interestingly, the equation for lateral diffusion through the *stratum corneum*, Eq. (16), also has a small positive v-coefficient. These positive coefficients could arise because the assumptions of the Stokes–Einstein equation, which predicts a negative v-coefficient for $\log Dc$, are not valid for intestinal absorption. The assumptions are: (1) the solvent medium is homogeneous; and (2) the solute molecules are larger than the solvent molecules. In addition, in the case of intestinal absorption, there could be other contributions that arise from transfer across the mucosal | membrane interface or possibly through diffusion through the membrane itself. Note that the slope of the van de Waterbeemd plot (Fig. 3) is always positive, which suggests a positive v-coefficient for the overall rate of transfer from water to an organic medium, where no membrane at all is involved.

4. Conclusions

There are a number of important consequences of our suggestion that in general the rate-determining step in human intestinal absorption may be diffusion through the stagnant mucosal layer together with transfer across the mucosal | membrane interface. First of all, there will be compounds for which this is not actually the rate-determining step. Such compounds will fall outside the scope of our Eq. (9), and outside the scope of the corresponding equation for % absorption. There will also be a number of sparingly soluble compounds for which the rate-determining step is that of dissolution of the solid, as discussed by Dressman et al. [4]. Hence in the correlation of % absorption with physicochemical descriptors, care must be taken that all the compounds in the data set used are absorbed by the same mechanism. Secondly, we have shown that water–solvent systems are not very suitable model systems for human intestinal absorption. Finally, and most importantly, the search for models for human intestinal absorption based on membrane systems that are characterised by either a partition coefficient or an overall rate of transfer through the membrane may not be very fruitful if the process to be modelled (i.e. human intestinal absorption) involves mainly diffusion through a stagnant mucosal layer as the rate-determining step.

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