J. Pharm. Pharmac., 1976, **28**, 572–579 *Received March* 8, 1976

A four parameter model for oral drug absorption

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A method has been developed for assessing absorption parameters from plasma concentration-time results taken over a relatively short time period. In this method, the disposition function is simplified so as to reduce the number of parameters to be evaluated from the five of the two compartment disposition equation to four, and to avoid the requirement for the evaluation of a slow disposition rate constant. The measurements need not therefore be continued over a period long after absorption is complete. A suitable design for kinetic experiments using this method for interpreting the results, is described. A random noise statistical method is proposed for assessing the stability of the calculated parameters.

Absorption with two compartment disposition

The mathematical model for first order drug absorption with two compartment disposition leads to a plasma concentration (C), time (T) equation with five parameters,

$$C = A.\exp(-\alpha.T) + B.\exp(-\beta.T) - (A + B).\exp(-k_a.T) \quad \ldots \quad (1)$$

There are too many parameters for reliable evaluation from experimental data by non-linear least squares. An alternative method based on the maximum point of the C,T curve has been proposed (Saunders & Natunen, 1973). However, this method requires determinations of plasma concentrations over a considerable period in order to define the slow disposition constant, β.

All the information related to absorption is contained in the first part of the C,T curve; at times later than twice the time, T_m, at which maximum C occurs, the absorption is generally more than 95% complete and it should therefore be possible to extract the absorption information from values of C determined over a limited period of time of the order of $2.T_m$. Westlake (1973) has discussed the desirability of defining limited objectives in pharmacokinetic studies and of concentrating experimental determinations in the time region where the parameters which are particularly required, have their main effect.

The use of limited time experiments is supported by the study made by Lovering, McGilveray & others (1975) in which they showed that relative bioavailabilities for a number of drugs assessed from areas under plasma concentration/time curves, could have been estimated with blood sampling over a 24 h period or less. The ratios of areas with a test and a standard formulation changed little between the end of the drug absorption period and the much later times at which blood sampling was terminated.

Many pharmacokinetic studies are concerned with the effects of formulation on drug absorption; it is then the parameters relating to absorption such as lag time and first order absorption rate constant which are of main interest. The object of the work described in this paper has been to develop a method for interpreting C,T results generated with equation (1) so that over a limited time period they may be interpreted by a simpler expression which preserves the absorption information.

An appropriate expression has been evolved and has been tested with a wide variety of generated C,T data. In some cases random noise to simulate experimental error in C has been added to check the stability of the calculations. Finally, it has been used with experimental C,T data.

A complete Fortran Programme to carry out all the necessary calculations has been written for the CDC computers of London University.

Development of a four parameter model

From equation (1), C may be considered to be the resultant of two functions, $A.\exp(-\alpha.T) + B.\exp(-\beta.T)$ related primarily to disposition and $(A + B).\exp(-k_a.T)$, a function related primarily to absorption; the values of the terms A and B are governed by both sets of rate constants; in Fig. 1 the two functions with the resultant C,T curve are shown for one set of data. It should be possible to express the first function with fewer than four parameters without unduly distorting the absorption function.

The simplest modification is to express the disposition as a single term $A_1.\exp(-k_d.T)$ where k_d

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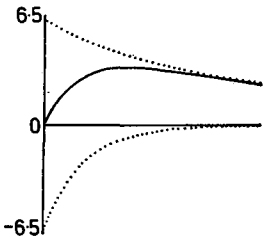

FIG. 1. Disposition and absorption functions for generated data Al,k_a = 0·8, up to 7 h. The top curve is A.exp(−α.T) + B.exp(−β.T); the bottom curve is −(A + B).exp(−k_a.T); the mid-curve is the resultant curve for C.

is an overall disposition constant (Saunders, 1975). The total equation is then,

$$C = A_1.(\exp(-k_d.T) - \exp(-k_a.T)) \quad .. \quad .. \quad (2)$$

In fact this equation, applied over a limited time period up to 2.T_m, gives good values of k_a with data generated by equation (1), for cases where k_a/k_d is large, as is seen in Table 1. When k_a and k_d are closer together distortion of the k_a values occurs and since much experimental data is in this region, a further parameter is needed in the disposition function to relieve the distortion.

When equation (2) is deduced from the rate equation, the value of A is found to be $X_0.k_a/(V.(k_a - k_d))$ where X_0 is the dose absorbed and V is the apparent volume of distribution. As k_d approaches k_a A becomes large and at the same time the differfence of exponential terms in equation (2) approaches the value $(k_a - k_d).\exp(-k_a.t)$, consequently the equation moves towards 0/0 and becomes unstable.

Many possible fourth parameters have been examined (Saunders, 1974) and we have found that the most effective one leads to the equation

$$C = A_2.(H + (1-H).\exp(-k_d.T) - \exp(-k_a.T)) \quad (3)$$

This equation maintains the condition C = 0 at T = 0 and the introduction of H, a positive constant, permits improved assessments of k_a from data generated with equation (1) up to T = 2.T_m.

Equation (3) cannot be taken to represent results generated by equation (1) over a long time period. At long times equation (3) approaches a limiting value of A_2.H and so does not give a decay of C at late times.

An analysis which is effective with theoretical data may break down when used with results subject to random experimental error. The calculation was therefore, tested with generated C,T values to which random noise simulating experimental error was applied to C.

Table 1. *Generated data. Al.* Input parameters k_1 = 0·1, k_2 = 0·1, k_e = 0·05, I = 100, α = 0·228, β = 0·0219. K_a = input absorption rate constant; C_m = maximum value of C; T_m = time at which the maximum occurs; CAL is type of calculation, 3φ is three ordinate. 4φ is four ordinate; k_a = estimated absorption rate constant; k_d, A_2 and H are the parameters of equation (4); L is the estimated availability; D is the sum of squared differences between input values of C and calculated values.

K_a	C_m	T_m	CAL	k_a	k_d	A_2	H	L	D
0·4	2·88	4·30	3φ	0·484	0·086	5·08			$4·10^{-4}$
			4φ	0·454	0·137	5·93	0·166		$9·10^{-5}$
			LSQ	0·418	0·183	7·50	0·173	4·21	$3·10^{-6}$
0·8	3·46	2·71	3φ	0·858	0·112	5·40			$3·10^{-4}$
			4φ	0·828	0·170	5·81	0·189		$5·10^{-5}$
			LSQ	0·804	0·203	6·18	0·226	4·61	$5·10^{-6}$
1·2	2·75	2·05	3φ	1·25	0·123	5·37			$2·10^{-4}$
			4φ	1·22	0·178	5·57	0·200		$3·10^{-5}$
			LSQ	1·20	0·208	5·73	0·251	4·73	$2·10^{-6}$
1·6	3·94	1·68	3φ	1·64	0·129	5·31			$1·10^{-4}$
			4φ	1·62	0·181	5·44	0·205		$3·10^{-5}$
			LSQ	1·60	0·210	5·53	0·263	4·80	$1·10^{-7}$
2·0	4·07	1·43	3φ	2·03	0·132	5·27			$2·10^{-4}$
			4φ	2·01	0·183	5·35	0·207		$2·10^{-5}$
			LSQ	2·00	0·211	5·41	0·271	4·83	$7·10^{-8}$
2·4	4·17	1·26	3φ	2·43	0·135	5·24			$1·10^{-4}$
			4φ	2·41	0·184	5·30	0·209		$2·10^{-5}$
			LSQ	2·40	0·212	5·34	0·276	4·86	$4·10^{-8}$
2·8	4·25	1·13	3φ	2·83	0·137	5·21			$1·10^{-4}$
			4φ	2·81	0·185	5·25	0·210		$2·10^{-5}$
			LSQ	2·80	0·213	5·29	0·280	4·88	$2·10^{-8}$

Method of calculation

The lag time before appreciable amounts of drug appear in the plasma was assessed by fitting a second degree polynomial to the first three points and extrapolating to $C = 0$ as already described (Saunders & Natunen, 1973).

The intercept, T_1 if positive was subtracted from all the experimental time values. The lag time itself is an interesting absorption parameter, related to the formulation of the drug.

The maximum point T_m,C_m was assessed by fitting a second degree regression to the points around the highest value of C in the data. Use of a regression and five points with C and log T as the variables gave a better method for flat maxima with data subject to random error, than the three point curve fitting procedure previously described (Saunders & Natunen, 1973). The use of log T allows for the asymmetry of the region around the maximum point.

Three ordinate calculation

In the first place the three parameter model of equation (2) was used, the fourth parameter H was then introduced in a subsequent calculation.

To avoid the necessity for providing starting values for the parameters k_a,k_d and A of equation (2), the equation was fitted to three ordinates C_1, C_m, C_2, at $T = T_m/2$, $T = T_m$, $T = 3T_m/2$. C_m had already been estimated and the values of the other two ordinates were assessed by interpolation with a second degree regression applied to the four nearest points when sufficient points were available or by a second degree curvilinear interpolation when the number of points was insufficient for the regression. The following scheme was then used.

$R_a = \exp(-k_a.T_m/2)$ $\quad R_d = \exp(-k_d.T_m/2)$

$C_1 = A.(R_d - R_a)$ $\quad C_m = A.(R_d^2 - R_a^2)$

$C_2 = A.(R_d^3 - R_a^3)$ $\quad Q_1 = C_m/C_1$, $Q_2 = C_2/C_1$

then $Q_1 = R_a + R_d$ $\quad Q_2 = R_a^2 + R_a.R_d + R_d^2$

and $R_a^2 - Q_1.R_a + Q_1^2 - Q_2 = 0$ .. .. (4)

the roots of this quadratic equation are R_a, the smaller, and R_d the larger. From their values A,k_a and k_d are assessed.

$$k_a = -(2/T_m).\ln(R_a) \quad k_d = -(2/T_m).\ln(R_d)$$
$$A = C_m/(R_d^2 - R_a^2)$$

The scheme broke down in cases where there were no real roots and this occurred when the estimated value of C_m was greater than can be expressed by equation (2) as was found with some scattered data. In such cases T_m was reassessed taking more points into the second degree polynomial and, if failure persisted, the calculation went straight to a four parameter least squares fitting with approximate values for the four parameters of equation (3)

$$k_a = 2/T_m, \quad k_d = k_a/2, \quad A = 3.C_m \quad H = 0$$

when k_a/k_d was greater than 10, the three parameter values of k_a were good estimates which were not much altered in subsequent calculations.

Four ordinate calculation

In all cases where real roots to equation (4) were obtained, the calculation was continued using equation (3) and bringing in a fourth ordinate, C_3, at $T = 2.T_m$. By a scheme modified from the 3 ordinate calculation, a value of H and consequently modified values of k_a,k_d and A were assessed giving a fit to the four ordinates. It was found best for this calculation to start with a moderately high value of H (0·3).

The scheme used was as follows. Starting with the 3 ordinate values of k_a, k_d and A with the above value for H.

$G_1 = C_1 - A_2.H.(1 - R_d) = A_2.(R_d - R_a)$ from equation (3)

$G_m = C_m - A_2.H.(1 - R_d^2) = A_2.(R_d^2 - R_a^2)$

$G_2 = C_2 - A_2.H.(1 - R_d^3) = A_2.(R_d^3 - R_a^3)$

$Q_1 = G_m/G_1 \quad Q_2 = G_2/G_1$

Q_1 and Q_2 then gave the same quadratic equation (4) as in the 3 ordinate method. Having reassessed R_d, R_a and A_2 with $H = 0{\cdot}3$, the value of H was recalculated from equation (3) at $T = 2.T_m$

$$C_3 = A.(H + (1 - H).R_d^4 - R_a^4)$$

C_3 was interpolated from the input data in the same way as C_1 and C_2. The whole set of calculations was repeated to a convergence of 0·001 in k_a.

Least squares calculation

The values so obtained were mostly a good representation of input absorption constants in generated data, however, when k_a and k_d were close, they were improved by a final non-linear least squares calculation with equation (3) taking the values from the 4 ordinate calculation as the starting values. The method of Gennrich & Sampson (1968) developed into the Biomedical Program BMD X85, was used. The least squares fitting was carried out with the original C.T data up to the point beyond $T = 2.T_m$ to give positive values of the four parameters. All the sets of data and the very small sums of squares,

indicated that equation (3) does give an excellent representation with respect to absorption, of data generated by equation (1).

In cases where there was greatest difficulty in fitting equation (3) the non-exponential parameter A_2 differed significantly from the input value of (A + B).

Generated data

C,T data was generated from the two disposition compartment model by giving a range of values to the rate contants k_a, k_1, k_2, k_e. The parameters of equation (1) are then given by:—

$$\alpha + \beta = k_1 + k_2 + k_e \qquad \alpha.\beta = k_2.k_e$$

All the sets of data were normalized so as to give a total area under the C, T curve of 100 units and about 14 points were estimated in the period up to $2.T_m$.

The parameters A and B of equation (1) may be expressed in terms of dose absorbed X_0, volume of distribution V and the rate constants. X_0/V may be replaced by $(k_e.I)$ where I is the total area under the C/T curve giving—

$$A + B = \frac{I.k_e.k_a.(k_a - k_2)}{(k_a - \alpha).(k_a - \beta)}$$

$$A = \alpha.[(A + B).(k_a - \beta)/k_a - \beta.I]/(\alpha - \beta)$$

Noise was applied by using a subroutine which generated random normal deviates. A coefficient of variation for the values of C was supplied as input together with the set of values of C and T. Sets of data were then generated containing random noise applied to C. Each set was analysed by the method described in the previous section and then means and standard deviations of the parameters for all the sets were estimated.

Availability

The availability of a drug following oral dosage is assessed by

$$X_0/V = k_e.I$$

X_0 = the amount of drug absorbed, V is the apparent volume of distribution, k_e is the elimination rate constant and I is the total area under the curve C,T. There is ambiguity about V and without some further information X_0 can only be expressed in terms of V. The corresponding expression from equation (3) is obtained by differentiating this equation and then substituting the value of $\exp(-k_d.T)$ from equation (3).

$$\frac{dC}{dT} = A_2.(k_a - k_d).\exp(-k_a.T) - k_d.(C - H.A_2)$$

The first term in this rate equation represents absorption while the second equation represents disposition. From this second term it is seen that the parameter H introduces a zero order component into the rate equation. The disposition rate is:—

$$\frac{1}{V}.\frac{d(X_e)}{dT} = k_d.(C - H.A_2)$$

X_e is the amount disposed at time T and V is the apparent volume of distribution.

The amount disposed up to $T = 2.T_m$ is

$$D_2 = V.k_d.\int_0^{2.T_m}(C - H.A_2).dT$$

$$= V.k_d.\left[\int_0^{2.T_m} C.dT - 2.H.A_2.T_m\right]$$

The integral is the area (I_2) under the C,T curve from $T = 0$ to $T = 2.T_m$.

If C_3 is the concentration in the plasma at $2.T_m$, the amount in the volume, V, at this time is $C_3.V$.

The total amount absorbed at time $2.T_m$ is then $X_2 = V.(k_d.(I_2 - 2.H.A_2.T_m) + C_3)$

The proportion of the dose remaining unabsorbed at $T = 2.T_m$ is $\exp(-2.k_a.T_m)$ and this is usually less than 5%. However, in order to give a good estimate of the availability of the drug, X_0 up to long times, the above expression is divided by $1 - \exp(-2.k_a.T_m)$

The final expression for availability then becomes

$$\frac{X_0}{V} = \frac{k_d.(I_2 - 2.H.A_2.T_m) + C_3}{1 - \exp(-2.k_a.T_m)}$$

With the generated data, values of availabilities (the generated data was all normalized to I = 100) were all somewhat below the input values of $100.k_e$.

RESULTS

Table 1 shows the results of calculation with a set of generated data giving disposition constants α = 0·228, β = 0·0219. The input absorption constant K_a was varied in steps of 0·4 from 0·4 to 2·8. All the rate constants are in h^{-1}; times in h.

In all cases except when K_a = 0·4, the estimated absorption constant k_a from the 3 parameter model was within 10% of the input value. The 4 parameter model improved the value of k_a and the fit to the data and the least squares calculation reduced the sum of squared deviations between input and calculated results to a very small value and at the

Table 2. *Generated data with fixed* k_a. $K_a = 0{\cdot}4$.

Input			Estimated from least squares with equation (3)					
k_1	k_2	k_e	k_a	k_d	A_2	H	L	k_e.I
0·1	0·1	0·05	0·418	0·183	7·50	0·173	4·21	5
0·15	0·1	0·05	0·425	0·236	9·09	0·135	4·02	5
0·1	0·1	0·1	0·424	0·212	17·3	0·088	8·66	10
0·15	0·1	0·1	0·437	0·259	20·2	0·078	8·20	10
0·1	0·1	0·15	0·429	0·248	31·0	0·048	13·1	15
0·01	0·1	0·1	0·400	0·205	20·2	0·012	9·85	10
0·02	0·1	0·1	0·400	0·208	20·3	0·022	9·59	10
0·04	0·1	0·1	0·404	0·214	20·1	0·040	9·45	10
0·06	0·1	0·1	0·409	0·216	19·5	0·057	9·18	10
0·09	0·1	0·1	0·419	0·214	18·0	0·080	8·79	10

same time improved the value of k_a, so that in the worst case k_a was estimated as 0·418 with an input value of 0·4.

The worst agreement between input and output absorption constants was found at the lowest k_a values with all sets of data. In Table 2, the sets of values with the same input K_a of 0·4 and varying 2 compartment rate constants are shown. The values of the 4 parameters k_a, k_d, A_2 and H are those from the final least squares calculation. The largest difference between input and output absorption constants is seen in the fourth row where k_a = 0·437. In this case $\alpha = 0{\cdot}32$, $\beta = 0{\cdot}031$. The value of α is close to K_a.

Table 3 indicates the time range of validity for the interpolation of concentrations generated from equation (1), by equation (3). With three sets of data it is seen that the first divergence in the second place of decimals occurs at around 3.T_m, with a 10% divergence between 5.T_m in set 1 and 10.T_m in set 2. At late times in all cases the values from equation (3) approach limiting values and are therefore greater than the C_1 values.

In Table 4 results are shown in which 10 sets of random noise simulating an experimental error of 3% in the concentration determination were applied to two sets of generated data. The calculations were successfully completed for all the sets of noise, giving a mean with the first set for k_a equal to 0·411 with a standard deviation of 0·071, that is a coefficient of variation of 17%. The other parameters showed large variations and in some cases the value of H reached the minimum of zero indicating that the scattered data could only yield three significant positive parameters.

The second set had a high input value of $k_a = 2{\cdot}8$. The mean with the noise sets was somewhat below this at 2·67 with a coefficient of variation of 14%. Some noise sets again gave zero H.

In Table 5 the results calculated with experimental values of prednisolone plasma concentrations following oral dosage are shown. These experimental results were published by Leclerq & Copinschi (1974) and are particularly suitable for analysis by this method since they contain a considerable number of results in the time period up to 2.T_m.

Table 3. *Comparison of concentrations from equations* (1), C_1, *and from equation* (3), *over a wide time range.*

Set 1. C_1 generated from I = 100, $K_a = 0{\cdot}4$, k_e = 0·15, $k_1 = 0{\cdot}1$, $k_2 = 0{\cdot}1$.
C_3 calculated from computer output values of rate constants of equation (3) with C_1 (up to 2.T_m) as the input $k_a = 0{\cdot}429$, $k_d = 0{\cdot}248$, $A_2 = 31{\cdot}0$, H = 0·0483, $T_m = 3{\cdot}268$.

Set 2. C_1 generated from I = 100, $K_a = 2{\cdot}8$, k_e = 0·15, $k_1 = 0{\cdot}1$, $k_2 = 0{\cdot}1$, C_3 calculated from $k_a = 2{\cdot}80$, $k_d = 0{\cdot}286$, $A_2 = 16{\cdot}5$, H = 0·117, $T_m = 0{\cdot}956$.

Set 3. C_1 from I = 100, $V_a = 0{\cdot}8$, $k_e = 0{\cdot}25$, $k_d = 0{\cdot}1$, $k_2 = 0{\cdot}1$, C_3 from $K_a = 0{\cdot}807$, $k_d = 0{\cdot}366$, $A_2 = 440$, H = 0·0347, T_m = 1·856.

Time is given as multiples of T_m.

Time	Set 1		Set 2		Set 3	
	C_1	C_3	C_1	C_3	C_1	C_3
0·25	3·77	3·77	7·08	7·08	7·12	7·12
0·5	5·81	5·81	10·30	10·30	10·98	10·98
0·75	6·75	6·75	11·57	11·57	12·76	12·76
1·0	7·00	7·00	11·87	11·87	13 25	13·25
1·5	6·47	6·47	11·29	11·29	12·23	12·23
2·0	5·46	5·46	10·27	10·27	10·27	10·27
2·5	4·46	4·46	9·25	9·25	8·28	8·28
3·0	3·62	3·63	8·33	8·33	6·57	6·58
3·5	2·96	3·00	7·51	7·52	5·22	5·24
4·0	2·46	2·54	6·79	6·80	4·18	4·23
5·0	1·80	1·98	5·61	5·64	2·81	2·93
6·0	1·40	1·72	4·70	4·75	2·03	2·24
8·0	0·95	1·54	3·44	3·56	1·28	1·71
10·0	0·67	1·51	2·66	2·87	0·93	1·57
12·0	0·48	1·50	2·15	2·48	0·71	1·54

Table 4. *Analysis with 3% random noise.*
Group Al. K_a = 0·4. Final values from the 4 parameter least squares calculation.

Set	k_a	k_d	A_2	H	L
1	0·418	0·182	7·48	0·173	4·21
2	0·485	0·087	5·06	0·03	4·16
3	0·474	0·089	5·28	0	4·30
4	0·373	0·225	11·3	0·128	4·48
5	0·287	0·193	17·9	0·035	3·86
6	0·358	0·174	9·62	0·086	4·97
7	0·496	0·082	4·93	0·5	4·15
8	0·510	0·083	4·98	0	4·15
9	0·365	0·208	10·7	0·117	4·63
10	0·379	0·210	9·99	0·133	4·47
11	0·378	0·345	44·1	0·044	3·71
Mean	0·411	0·171	11·9	0·068	4·28
Std	0·071	0·081	11·3	0·062	0·349

Group A1. K_a = 2·8.

Set	k_a	k_d	A_2	H	L
1	2·80	0·213	5·29	0·281	4·88
2	2·83	0·128	5·14	0	4·82
3	2·75	0·140	5·21	0	4·92
4	2·01	1·24	11·4	0·311	4·35
5	3·07	0·115	4·99	0	4·72
6	2·87	0·136	5·21	0	4·95
7	2·68	0·158	5·44	0	5·1
8	2·00	1·18	11·5	0·291	4·67
9	3·03	0·124	5·05	0	4·69
10	2·47	0·899	7·22	0·454	4·62
11	2·71	0·445	5·63	0·499	4·74
Mean	2·665	0·435	6·55	0·166	4·77
Std	0·361	0·450	0·250	0·202	0·198

The plasma determinations were irregular for most of the individual results, the analysis was therefore carried out with the mean of the 10 sets of results reported in the paper. The 4 parameter calculation improved the fit compared with the 3 parameter calculation, but this was not much improved by the least squares calculation. In the 3 calculations k_a moved from 0·88 to 1·22 and back to 0·99. When 3% random noise was applied, a mean k_a of 0·79 with a coefficient of variation of 39% resulted, so that this set of data is somewhat unstable to noise. The best estimates of the absorption parameters are lag time = 0·19, absorption constant = 0·99, availability 191.

A similar calculation was made with data published in the above paper for mean plasma concentrations of prednisolone following dosage with enteric coated tablets. The effect of the enteric coating was apparent with a lag time of 0·87 h and a later time for the peak concentration (2·90 h). These results did not give values with the 3 and 4

Table 5. *Mean prednisolone (20 mg tablets).*

C 15·0, 70·0, 116, 129, 148, 162, 160, 155, 150, 146, 143 ng ml^{-1}
T 0·25, 0·50, 0·75, 1·00, 1·25, 1·50, 1·75, 2·00, 2·50, 3·00, 3·50 h

Calc.	Cm	Tm	k_a	k_d	A_2	H	L	D
30	161	1·45	0·885	0·453	666			4.10^{-2}
40			1·22	0·478	3482	0·278		2.10^{-2}
LSQ			0·987	0·915	2645	0·045	191	$1·5.10^{-2}$

With 3% random noise.

	k_a	k_d	A_2	H	Cm	Tm
Mean	0·792	0·588	1762	0·072	158	1·69
Std	0·311	0·221	1252	0·133	3·09	0·179

ordinate calculations, the quadratic equation having no real roots. The least squares calculation starting with the approximate set of values for the 4 parameters gave k_a = 0·35 with zero H, availability = 284. The results with 3% noise were mean k_a = 0·37, coefficient of variation 9% indicating a reasonably stable calculation.

Availabilities

The estimates of X_0/V are called L in the Tables. In Table 1 they are seen to be somewhat below the theoretical value for the input data of (k_e.I) = 5·0, the smallest value being with the first set, L = 4·2. This is the set which gives the worst k_a estimate.

In Table 2, L is again below the input values of k_e.I the worst value in percentage terms is 4·02 with an input of 5·0.

In Table 4 it is seen that L is relatively stable to random noise applied to C.

From the prednisolone results the plain tablets gave an availability of 191 (ng ml^{-1}) while the enteric coated tablets showed a higher value of 284 corresponding to the longer time period over which high plasma concentrations are sustained.

These availability estimates are necessarily approximate but they should be helpful in assessing relative values for differing formulations.

Design of experiments

To apply the methods of calculation outlined in this paper it is important to design the experimental plasma concentration determinations so that they are spread as uniformly as possible over the time range 0 to 2.T_m. There should be about 5 determinations between O and T_m and a further 5 from T_m to 2.T_m. One or two results should be determined at times shortly after 2.T_m.

J. Garnham and K. Raymond have made available to us some sets of plasma concentration-time data for the absorption of a non-acidic anti-inflammatory substance in man after dosage with 500 mg tablets. The measurements were not designed for this method of interpretation; one set of results which came nearest to the design outlined above had 7 results in the period up to $T = T_m$, 2 results between T_m and $2.T_m$ and one result after $2.T_m$. These results are given in detail in Table 6. T_l was 0·083 h and T_m was 7·15 h. The 3 ordinate calculation gave a value of k_a of 0·26 h^{-1} which was modified by the introduction of H to 0·245. The least squares method gave further modification of k_a to 0·180. The value of the sum of squared deviations of calculated from experimental concentrations was not reduced to a very small value owing to the irregularity in the late time results, a situation which is always liable to occur with experimental data.

Table 6. *Absorption parameters for the anti-inflammatory agent.*

T	0·5	1·0	1·5	2·0	3·0	4·0	6·0	8·0	12·0	24·0	h
C	0·5	1·0	1·7	2·7	4·5	6·6	6·9	6·4	6·5	3·0	μg ml^{-1}

	k_a	k_d	A_2	H	L
3φ	0·256	0·063	14·59		
4φ	0·245	0·083	16·55	0·0910	
LSQ	0·180	0·127	38·92	0·0496	13·1
9φLSQ	0·190	0·114	30·75	0·0596	12·4

On plotting the theoretical functions for the 4-ordinate and least squares calculations for this set of results, as shown in Figs 2 and 3, it was apparent that the least squares method gave a much inferior visual fit to the results than the former. This effect was caused by the slight concavity of the early results which meant that in unweighted least squares terms it was expensive for the function to miss these points too widely, causing a poor representation of the important points around the maximum.

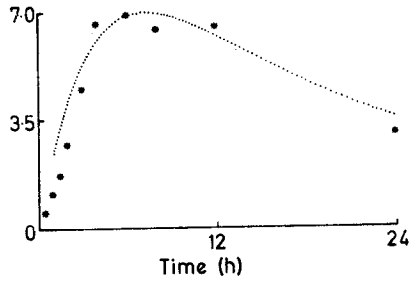

FIG. 2. Data in Table 5 for an anti-inflammatory agent with curve representing equation (3) using parameters from 4φ calculation. C in μ ml^{-1} T in hours.

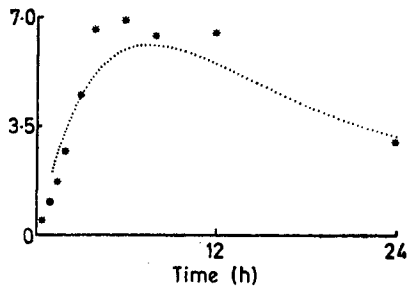

FIG. 3. Same data as Fig. 2 curve from LSQ calculation.

As a consequence of these results, a further option has been introduced into the programme for use with experimental results which do not fit the experimental design. In this option nine values of C are interpolated from the set of experimental results with values of T at invervals of $T_m/4$ from $T_m/4$ to $9T_m/4$. The least squares calculation is then performed on these nine results so ensuring a uniform weighting over the time interval.

This 9-ordinate method made little difference to the calculations for the prednisolone example where the points used were well spread across the interval, however, it gave a much better visual fit to the results for the anti-inflammatory agent, as is shown in Fig. 4. The estimated parameters by the different methods are given in Table 6.

It is difficult to gauge the taking of plasma samples to fit an exact experimental design, particularly as T_m is likely to vary between subjects. The 9 ordinate interpolation option can be used to give a balanced least squares calculation over the significant time period.

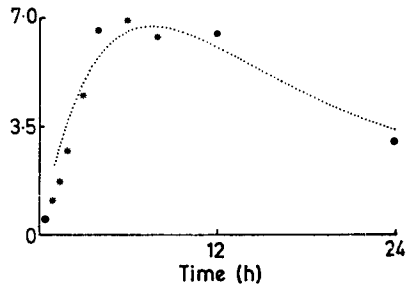

FIG. 4. Same data as Fig. 2 curve from 9φLSQ calculation.

In their unpublished work, Garnham & Raymond made studies at three dose levels, 500, 1000 and 1500 mg. It was of interest to see how the availability estimate L varied with the dose. Individual subject results which were interpretable by equations (2) and (3), showed considerable variation in L. At the 500 mg dose, the results were 5·2, 12·4, 20·2, 17·5, for 4 subjects; at the 1000 mg dose, 22·6, 17·9, 18·9, 36·5 and at the 1500 mg dose, 24·0, 41·2, 43·7. The means of these results do reveal a significant correlation with dose; 500 mg dose, mean L = 13·8; 1000 mg dose, mean L = 24·0; 1500 mg dose, mean L = 36·3 μg ml^{-1}.

Absorption parameters and statistical considerations

The quantities related to drug absorption which result from these calculations are lag time, absorption rate constant and availability. They should provide information for comparing formulations in groups of subjects.

The noise method may be used to assess the stability of the calculated parameters. For this purpose a coefficient of variation for the analytical method used should be found by repeated determinations on appropriate plasma samples.

In favourable cases where the coefficients of variation of the parameters are reasonably small, it should be possible to assess whether a significant difference between formulations has been established. The noise method may indicate that the estimates of absorption parameters have large coefficients of variation which render their quantitative significance small.

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