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A kinetic study of human urinary excretion results for butobarbitone and its metabolites

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Experimentally determined concentrations of butobarbitone, and three or its metabolites, in the urine of two volunteers, have been studied using a computer technique, which tests the data for limited capacity or first order kinetics. In both volunteers, the 3′-hydroxy and the 3′-oxo metabolites follow limited capacity kinetics for the first few days of excretion, and unchanged butobarbitone follows first order kinetics. Differing results were found for the two persons with the 3′-carboxylic acid metabolite. An additional calculation is proposed when applying the Levy method for interpreting limited capacity kinetics to data where drug recovery is incomplete.

The interpretation of urinary excretion results is best made in terms of cumulative excretions at the end of defined time periods. The experimental measurements are usually subject to considerable random error and it is unlikely that any very meaningful results are obtainable for absorption (KA) or fast disposition rate constants (α) from urine excretion results alone. However, the slow disposition constant (β of the two compartment disposition theory) may be estimated and, in addition, the kinetic types of the excretion processes of the unchanged drug and metabolites may be evaluated. A method developed by Levy, Tsuchiya & Amsel (1971) has been used to determine whether the excretion can be considered as first order or whether it is a limited capacity process requiring interpretation by the Michaelis-Menten equation.

From the experimental results, S, the amount of drug remaining in the body and the rates of excretion R of each species are evaluated at each time. If the excretion is first order the ratio S/R should be constant showing no significant variation with S; if it involves limited capacity effects, S/R has a significant positive linear correlation with S.

For first order kinetics, if Y is the amount of unchanged drug or of a metabolite excreted at time T and R is the rate of excretion

$$R = \frac{dY}{dT} = K1.S$$

$$\therefore \frac{S}{R} = \frac{1}{K1} \qquad \ldots \quad \ldots \quad \ldots \quad (1)$$

K1 is therefore the reciprocal of the mean value of S/R

For Michaelis-Menten kinetics

$$R = \frac{KM.RM.S}{1 + KM.S.}$$

RM is the maximum value of R

$$\frac{S}{R} = \frac{1 + KM.S}{KM.RM}$$

and KM is related to the second Michaelis-Menten constant

$$\frac{S}{R} = \frac{1}{KM.RM} + \frac{1}{RM} \cdot S \qquad .. \quad .. \quad .. \quad (2)$$

S/R is therefore a linear function of S. If S/R gives a significant linear correlation with S, the limited capacity kinetic equation is used. RM the maximum rate for the process is then equal to the reciprocal of the slope of the regression line of (S/R) and S, and KM is the ratio of the slope to intercept; in cases where S/RM is small, the Michaelis-Menten equation (2) merges into the first order equation (1).

METHODS

Butobarbitone was given orally to each of two healthy male volunteers as 2 × 100 mg butobarbitone tablets B.P., before retiring. Urine was collected in 12 h batches over several days and the samples were analysed for unchanged drug and three metabolites which have been identified as 3′-hydroxyl, 3′-oxo and 3′-carboxylate derivatives, using gas chromatography-mass spectrometry (Gilbert & Powell, 1974).

Outline of the calculations

The amounts of unchanged drug X1 and metabolites X2, X3, X4 were calculated from the experimental results as molar percentages of the dose excreted during each time period. The summed excretion in each time interval is then the sum XTOT = X1 + X2 + X3 + X4.

In order to assess S, the amount in the body at any time, it is necessary to find the total amount excreted; even after 8 days detectable amounts were still appearing and so this quantity was determined by extrapolation. If Z is the sum of increments XTOT up to time T, then the last three values of Z at the latest times are plotted against 1/T and extrapolated to 1/T = 0, that is, infinite time. The intercept ZO is taken as the total amount of drug which is determinable by the methods used. Values of S at each time are then given by:

$$S = ZO - Z \qquad .. \quad .. \quad .. \quad (3)$$

In cases where drug recovery is incomplete as with barbiturates (Maynert 1965), this extrapolated value is the best information available for the calculation of S. The consequent uncertainty in S will be more pronounced at late times; it is not likely to affect the discrimination between first order and limited capacity kinetics at the earlier times, as is shown by the results given in the Appendix.

Rates of appearance of each excreted species are calculated as follows. Values of Y the total amount of a single species excreted up to time T are found by summing the values of X up to that time. In the first time period there is usually evidence of a lag period and the lag time is assessed by taking the first three values of Y at the shortest times, fitting a second degree polynomial to them and extrapolating to Y = 0; the intercept on the T axis, if positive, is taken as the lag time, TLAG. When the second point is below the straight line joining the first and third points a linear extrapolation through points one and three is used for TLAG.

The rates of appearance of unchanged drug and of each metabolite in the urine at the end of each time period are assessed as follows. If X(I) is the amount excreted from T(I − 1) to T(I) and X(I + 1) is the amount in the interval T(I) to T(I + 1) then the rate at T(I) is estimated as

$$R = \frac{X(I)}{T(I) - T(I-1)} + \frac{T(I) - T(I-1)}{T(I+1) - T(I-1)} \cdot \left[\frac{X(I+1)}{T(I+1) - T(I)} - \frac{X(I)}{T(I) - T(I-1)} \right] \quad (4)$$

This expression accommodates differing time intervals. In the first period the time interval is taken as from TLAG to T(1).

With the values of S and R for each metabolite the test for first order versus saturable kinetics is made by calculating the correlation coefficient of (S/R) with S. If this coefficient is less than the tabulated value for $P = 0{\cdot}95$ and degrees of freedom equal to number of points minus two, first order kinetic theory is presumed and values of S/R are averaged to give a mean rate constant; if it is greater than the tabulated value, limited capacity kinetic theory is presumed and the slope and intercept of the (S/R) on R regression line are used to calculate the constants of equation (2).

At the later times the function S/R becomes somewhat uncertain and tends, in all cases examined, to increase as S decreases. Limited capacity kinetic effects are more likely to be observable in the early time (large S) region and so a scheme has been developed to explore the S/R, S data in groups of 4 points starting from the earliest, to test for significant correlation.

In this scheme, the S/R, S points 1 to 4 (at the shortest times) are tested against the tabulated correlation coefficient for 2 degrees of freedom. If the correlation is significant further points are added until significance is lost. If the correlation is not significant points 2 to 5 are taken and the procedure repeated. In this way any possible appearance of saturation is explored.

Finally, calculated values for the experimentally determined quantities are assessed using mean rates over the time intervals. Where limited capacity effects appear, the calculated increments of unchanged drug and metabolites may be made from both Michaelis-Menten and first order constant using equations (1) and (2), giving a further assessment of the significance of the saturation. The fit between theory and experiment is expressed as a dimensionless quantity RMSΔ.

$$\text{RMS}\Delta = \frac{\sqrt{\Sigma(\text{XCAL} - \text{X})^2/\text{N}}}{\Sigma(\text{X})/\text{N}} \quad .. \quad .. \quad .. \quad (5)$$

This quantity is the root mean average squared difference between calculated and observed values of X, divided by the mean value of the results.

Total excretion

If total excretion is considered overall as a first order process from the plasma, then

$$\frac{dS}{dT} = -\text{KE.D.C}$$

D is the volume of distribution. Even if the overall disposition follows a biexponen-

tial equation, at nearly all the times considered the plasma concentration C will be in the slow disposition (β) phase

$$C \sim B.\ \exp(-\beta.T)$$

$$\frac{dS}{dT} = -KE.D.B.\exp(-\beta.T)$$

$$S = KE.D.B.\exp(-\beta.T)/\beta$$

(since S = 0 at infinite time, the integration constant is zero). Consequently a plot of ln(S) against T should be linear with slope $-\beta$. From the regression ln(S) on T a value for the slow plasma disposition constant β, may therefore be found.

RESULTS

The units used to express the results are, times in hours, quantities of substance X, Y, Z, S as molar percentage of the dose of 200 mg, VM, as % h^{-1} and K1 and β as h^{-1}; the second limited capacity constant KM has the dimensions of reciprocal S.

Case 1

The experimental results are shown in Table 1. The last three results in the last column were regressed upon 1/T and extrapolated to 1/T = 0 to give ZO = 59·86. Values of S were then calculated as (ZO − Z).

The slope of the regression ln S on T gave a value of β of 0·0125 h^{-1} corresponding to a slow disposition half-life of 55·5 h.

Rates were calculated for each time and for each metabolite and the correlation of S/R with S was assessed. The thirteen points in Table 1 gave 12 rates and so 10 degrees of freedom in the overall correlation coefficient.

A summary of the results of these calculations is given below.

Table 1. *Experimental results for Case 1.*

T	Butobarbitone X1	3′-Hydroxyl X2	3′-Oxo X3	3′-Acid X4	Total XTOT	Cumulative total Z
12	0·75	1·69	0·35	0·51	3·30	3·30
24	1·00	3·48	1·39	0·83	6·70	10·00
36	0·96	3·74	2·47	0·90	8·07	18·07
48	1·08	3·78	1·32	0·76	6·94	25·01
60	0·41	2·52	2·36	0·48	5·77	30·78
72	0·80	2·90	1·34	0·49	5·53	36·31
84	0·30	1·81	0·83	0·34	3·28	39·59
96	0·38	1·62	2·17	0·31	4·48	44·07
108	0·21	1·13	0·51	0·32	2·17	46·24
132	0·50	2·03	0·43	0·40	3·36	49·60
156	0·30	1·23	0·57	0·22	2·32	51·92
180	0·22	0·69	0·13	0·11	1·15	53·07
204	0·12	0·35	0·18	0·052	0·702	53·77
TLAG	6·76	6·38	9·82	4·92		
	ZO = 59·9		$\beta = 1{\cdot}25 \times 10^{-2}$		Half life = 55·5	

Butobarbitone. TLAG = 6·76. Correlation coefficient (CC) −0·725; tabulated value (CT) 0·576. A test of successive points in groups of four showed no significant

positive correlations. The first order constant for the excretion of butobarbitone was calculated as the reciprocal of the mean value of S/R, $1{\cdot}58 \times 10^{-3}$, coefficient of variation (CV) 6%.

3'-Hydroxyl metabolite. TLAG = 6·38; points 1 to 8, up to 96 h, showed a significant positive correlation indicating limited capacity kinetics. CC was 0·755 compared with CT = 0·707. The constants of equation (2) were RM = 0·837, KM = 86·35. An alternative overall first order interpretation gave K1 = $6{\cdot}11 \times 10^{-3}$, CV = 35%. RMSΔ for the 8 points was 0·138 by the limited capacity theory and 0·204 by first order theory.

3'-oxo metabolite. TLAG = 9·82; points 1 to 8 showed a significant positive correlation, CC = 0·911, CT = 0·707. Regression analysis gave Michaelis-Menten constants of RM = 0·209, KM = 16·2. Overall first order analysis gave a rate constant of $2{\cdot}72 \times 10^{-3}$, CV = 19·6%. The fits to the first eight points gave for Michaelis-Menten theory RMSΔ = 0·365, for first order RMSΔ = 0·544.

3'-Acid metabolite. TLAG = 4·92; CC = −0·462; CT 0·576. No significant positive correlations were found. First order rate constant $1{\cdot}19 \times 10^{-3}$, CV = 14%.

Table 2. *Comparison of Michaelis-Menten and first order interpretations for the 3'-hydroxyl metabolite, Case 1.*

T	Experimental X2	Michaelis-Menten X2	First order X2
12	1·69	1·86	1·94
24	3·48	3·82	3·90
36	3·74	3·47	3·36
48	3·78	3·08	2·81
60	2·52	2·71	2·34
72	2·90	2·34	1·93
84	1·81	2·03	1·61
96	1·62	1·73	1·32
RMSΔ		0·138	0·204

Table 3. *Theoretical interpretations for Case 1.*

	Butobarbitone		3'-Hydroxyl		3'-Oxo		3'-Acid	
T	X1(exp)	X1(calc)	X2(exp)	X2(calc)	X3(exp)	X3(calc)	X4(exp)	X4(calc)
	First order		Limited capacity		Limited capacity		First order	
12	0·75	0·47	1·69	1·86	0·35	0·35	0·51	0·48
24	1·00	1·00	3·48	3·82	1·39	1·92	0·83	0·76
36	0·96	0·87	3·74	3·47	2·47	1·84	0·90	0·66
48	1·08	0·73	3·78	3·08	1·32	1·76	0·76	0·55
60	0·41	0·61	2·52	2·71	2·36	1·66	0·48	0·46
72	0·80	0·50	2·90	2·34	1·34	1·55	0·49	0·38
84	0·30	0·41	1·81	2·03	0·83	1·44	0·34	0·31
96	0·38	0·34	1·62	1·73	2·17	1·32	0·31	0·26
108	0·21	0·28					0·32	0·21
132	0·50	0·45					0·40	0·34
156	0·30	0·34					0·22	0·26
180	0·22	0·28					0·11	0·21
RMSΔ	0·293		0·138		0·365		0·340	

The negative values for CC found for the overall correlations were due to high values of S/R at late times (low S).

The appearance of limited capacity kinetics with the 3′-hydroxyl metabolite was explored further by comparing experimental values of X with calculated values for points 1 to 8 found from both Michaelis-Menten and first order rate constants; the results are shown in Table 2.

The Michaelis-Menten interpretation gives a better interpretation for these points than does the first order theory.

The fit of the theoretical interpretations to the experimental results is shown in detail in Table 3.

Case 2

In this case the measurements were taken over a shorter period and therefore the extrapolation for ZO is somewhat less definitive than in Case 1. The experimental results are shown in Table 4.

Table 4. *Experimental results for Case 2.*

T	Butobarbitone XI	3′Hydroxyl X2	3′Oxo X3	3′-Acid X4	XTOT	Z
12	0·58	1·33	0·04	0·33	2·28	2·28
24	2·03	2·95	1·59	0·66	7·23	9·51
36	1·53	3·46	3·44	0·68	9·11	18·62
48	1·40	3·19	2·44	0·76	7·79	26·41
60	1·06	3·51	3·06	0·75	8·38	34·79
84	1·15	3·60	3·89	0·66	9·36	44·15
108	0·83	2·66	1·85	0·39	5·73	49·88
TLAG	7·70	7·02	11·81	6·09		

ZO = 68·5 $\beta = 1{\cdot}38 \times 10^{-2}$ half life = 50·2

The results of the (S/R) with S correlations were as follows.

Butobarbitone. No significant positive correlations, mean first order rate constant $2{\cdot}206 \times 10^{-3}$, CV 6·5%.

3′-Hydroxyl. Significant positive correlation for points 1 to 5, that is up to 60 h, giving Michaelis-Menten constants, RM = 0·270, KM = 0·732. Comparison of Michaelis-Menten and first order interpretations up to 60 h indicated that the former gave a better fit to the observed results.

3′-Oxo. Significant positive correlation for points 2 to 5, the Michaelis-Menten interpretation was better than the first order for these points. RM = 0·189, KM = 7·21.

3′-Acid. This metabolite showed evidence of saturable kinetics and the results are given below in detail. The significant positive correlation was for points 1 to 5 up to 60 h. The correlation coefficient was 0·970 compared with the tabulated value of 0·878. Michaelis-Menten constants were RM = 0·0567, KM = 0·167. The overall first order constant was $1{\cdot}085 \times 10^{-3}$.

Table 5 shows a comparison between the experimental results for this metabolite and values calculated by the two theories.

Table 5. *Comparison of Michaelis-Menten and first order interpretations for the 3'-acid metabolite, Case 2.*

T	Experimental	Michaelis-Menten	First order
12	0·33	0·33	0·42
24	0·66	0·68	0·82
36	0·68	0·68	0·71
48	0·76	0·68	0·60
60	0·75	0·68	0·49
RMSΔ		0·078	0·248

DISCUSSION

The appearance of evidence for limited capacity kinetics for all metabolites in Case 2 but for only two of these in Case 1 may be connected with the higher apparent absorption in Case 2 as indicated by the extrapolated total percentage recovery of dose in the urine (ZO) which was 68·3% in Case 2 and 59·9% in Case 1. The lag times for unchanged drug and all three metabolites were higher in Case 2 than in Case 1 indicating a slower onset of absorption.

In Case 2 the evidence seemed clear that the 3'-hydroxyl, 3'-oxo and 3'-acid metabolites were all excreted by processes which up to 60 h, were capacity limited. In Case 1 3'-hydroxyl and 3'-oxo metabolites showed this Michaelis-Menten effect. In both cases the 3'-oxo metabolite was slower to appear in the urine than the other two with longer lag times indicting a slower and more complex oxidation mechanism for this substance than for the other two metabolites.

A summary of the kinetic parameters for both cases is given in Table 6. The coefficients of variation for the limited capacity kinetic constants were reasonable for RM but in some cases they were very large for KM, indicating that the numerical values for this parameter are uncertain, as is seen from the wide spread of KM values in Table 6.

The recognition of limited capacity excretion processes is important in considering plasma levels following repeated doses of a drug. Tsuchiya & Levy (1972) have discussed the possibilities of adverse and toxic consequences arising from multiple dosing, due to this effect.

Table 6. *Summary of kinetic parameters.*

	Case 1	*Case 2*
Apparent slow disposition constant β	$1{\cdot}25 \times 10^{-2}$	$1{\cdot}38 \times 10^{-2}$
Half life	55·5	50·2
Extrapolated total excretion, ZO	59·9	68·5
Lag times		
Butobarbitone	6·76	7·70
3'-Hydroxyl	6·38	7·02
3'-Oxo	9·82	11·81
3'-Acid	4·92	6·09
Averaged first order constants: K1		
Butobarbitone	$1{\cdot}58 \times 10^{-3}$	$2{\cdot}21 \times 10^{-3}$
3'-Hydroxyl	$6{\cdot}11 \times 10^{-3}$*	$5{\cdot}32 \times 10^{-3}$*
3'-Oxo	$2{\cdot}72 \times 10^{-3}$*	$4{\cdot}47 \times 10^{-3}$*
3'-Acid	$1{\cdot}19 \times 10^{-3}$	$1{\cdot}085 \times 10^{-3}$*
Limited capacity constants; RM, KM		
3'-Hydroxyl	0·837, 86·4	0·270, 0·73
3'-Oxo	0·209, 16·2	0·189, 7·21
3'-Acid	—	0·0567, 0·167

* The asterisk denotes that initially the limited capacity, Michaelis-Menten, theory gives better results than the first order theory.

In the Appendix, the results of repeating the calculations using 100 in place of ZO and assuming a decay of the unrecovered percentage of the drug (100−ZO) as a first order process with a mean rate constant equal to β, are described. This calculation does not affect the kinetic type of any of the excretions; first order constants are decreased by a factor roughly equal to ZO/100.

Computer program

A program to make the calculations described in this paper has been written in Fortran IV for CDC computers. On this program, excretion results for unchanged drug and up to nine metabolites are the input. These results are in the form of molar percentages of the dose excreted in defined but not necessarily equal, time intervals. The authors will be pleased to supply microfilm or line printer copies of the program together with an outline of its operation.

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APPENDIX

Levy method with incomplete drug recovery

In both cases considered in the paper, drug recoveries estimated by extrapolation to infinite time were incomplete. The incomplete recovery could be due to several causes:

(i) excretion by routes other than the urine,

(ii) formation of hydrophilic metabolites which would not have been detected by the analytical method used,

(iii) firm absorption into poorly perfused fatty tissue.

In order to test the effect of the unrecovered drug on the conclusions reached concerning the nature of the metabolite excretion processes, the program was re-run with the two sets of data, adding to the values of S a term representing the unrecovered drug undergoing a first order decay process with the overall rate constant β, calculated from the data

$$S' = S + (100-ZO)\exp(-\beta.T)$$

The results of these calculations are summarized in Table A1. The kinetic types of each excretion are unchanged, first order constants are changed by a factor roughly equal to 100/ZO; limited capacity maximum rates are of the same order as in Table 4, in one case the constant KM is substantially changed. However, as already noted the estimates of this quantity are very uncertain and show wide variations.

The correct values for rate constants should lie between the limits indicated by the two methods of calculation. The method used in the paper assumes that only ZO % of the dose is involved in the kinetic processes studied, the method outlined in the Appendix assumes that all the dose is involved and that an overall decay constant equal to that found for unchanged drug and the metabolites detected, governs the kinetics of the unrecovered drug.

The program can be modified to make this latter calculation by the addition of a single statement.

$$S(I) = S(I) + (100-ZO).\exp(-\beta.T(I))$$

and in cases of incomplete recovery both methods should be used so giving limits for the rate constants.

Table A1. *Kinetic parameters with S'.*

	Case 1	*Case 2*
Averaged first order constants; K1		
Butobarbitone	0.94×10^{-3}	1.58×10^{-3}
3′-Acid	0.71×10^{-3}	
Limited capacity constants; RM, KM		
3′-Hydroxyl	0·52, 60·1	0·27, 0·79
3′-Oxo	0·16, 1·2	0·19, 10·2
3′-Acid		0·056, 0·48