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MEAN SOLUTE CONCENTRATION FOR USE WITH THE MICHAELIS-MENTEN EQUATION APPLIED TO THE ANALYSIS OF DATA FROM INTESTINAL PERFUSION EXPERIMENTS

GORDON L. ATKINS

Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh EH8 9XD (U.K.)

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A number of algebraic expressions for the solute concentration for use with the Michaelis-Menten equation during the analysis of data from intestinal perfusion experiments have been investigated. It is concluded that the most suitable, especially if water absorption is occurring, is of the form: $s = (s_{\text{initial}} - s_{\text{effluent}})/\ln(s_{\text{initial}}/s_{\text{effluent}})$.

The Michaelis-Menten equation, originally proposed for use in enzyme kinetics, has often been fitted to data obtained for the rate of solute absorption during intestinal perfusion experiments. For this application it takes the form

$$\text{rate of absorption} = V \cdot s/(K + s)$$

where s is a solute concentration and K and V are parameters to be estimated (see, for example Ref. 1). A major difficulty however is to know what value of s to use in the equation since the solute concentration is not constant and forms a gradient down the intestine as solute is progressively absorbed. Should s be the initial concentration in the perfusate (s_0), the effluent concentration (s_n), the arithmetic mean of the two (s_a), the geometric mean of the two (s_g) or some other empirical function? Unfortunately the differential equations describing solute and water absorption rates along the length of the intestine cannot be integrated analytically and an approximation, albeit empirical, must be used. In a previous simulation study [2] I concluded that an empirical function for s of the form

$$s_e = (s_0 + s_n + 4.0\sqrt{s_0 \cdot s_n})/6.0$$

was the best one to use if K and V were to be well determined over a wide range of initial solute concentrations and perfusion rates.

Fisher and Parsons [1], faced with the essentially similar problem of fitting the Michaelis-Menten equation to data for intestines in a continuously recirculated closed system in which the solute concentration was changing, used an 'integrated mean' value for s thus

$$s_i = (s_0 - s_n)/\ln(s_0/s_n)$$

Winne et al. [3] have subsequently used this function for the analysis of data from single pass perfusions and it was suggested that I should extend my previous study to include this 'integrated mean' value (Winne, D., personal communication). Although Winne et al. [3] have successfully used this function it must be stressed that the goodness of fit of real experimental data does not provide a crucial test of the validity of this approach, since one than has to assume that the basic Michaelis-Menten equation (or a variation of it) is valid. This assumption is not necessarily valid, as discussed by Atkins and Gardner [4].

Winne and Markgraf [5] have argued that the 'integrated mean' value did not necessarily provide a suitable value for s if water was simultaneously being absorbed. It would be valuable to know, therefore, whether this empirical function is valid for models in which the rate of water absorption along the length of the intestine is either (i) constant, (ii) proportional to solute concentration, or (iii) proportional to solute absorption rate. Models (ii) and (iii) are physiologically more plausible than model (i). In my previous study I fitted my empirical function (s_e) to data from model (i), but not the other two. It would therefore be also valuable to extend the survey of my function to include these situations.

Details of the preparation of the data sets and the method for fitting the Michaelis-Menten equation may be found in the earlier study [2]. Water absorption rate, when proportional to solute concentration, equalled $(0.0027/s_0) \cdot s$. When proportional to solute absorption rate, the water absorption rate equalled $10.8(\Delta s/\Delta t)$. These functions were chosen so that when $K = 16.0$ µmol/ml and $V = 0.463$ µmol · min^{-1} · cm^{-1} the amount of water absorbed was similar to that observed by Fisher and Gardner [6] during their perfusion of isolated rat intestines.

The results, using the ten sets of data with $K = 16.0$ µmol/ml, $V = 0.463$ µmol · min^{-1} · cm^{-1}, a constant rate for water absorption and the perfusion rate (V_p) equal to 1.0, 2.0, ... 9.0, 10.0 ml/min, were looked at in more detail. At each of the ten perfusion rates the use of s_0 and s_n produced errors in K greater than 5%. The use of s_a and s_g produced errors in K greater than 1% at $V_p = 1.0$ and 2.0 ml/min only. Using s_i caused errors in K greater than 1% at $V_p = 1.0$ ml/min and using s_e, K was always precise within 0.2%.

Table I provides a summary of all the results for K and V obtained in this study when they were estimated by fitting the Michaelis-Menten equation to perfect data using, as the independent variable, the six algebraic functions for solute concentration. The models from which the data were generated had $K = 1.6$, 16.0 or 160.0 µmol/ml; $V = 0.0463$, 0.463 or 4.63 µmol · min^{-1} · cm^{-1}; and water absorption along the length of the intestine was either constant, or proportional to substrate concentration or proportional to solute absorption rate. Each model was used at ten different perfusion flow rates. The maximum total number of parameter estimates (along the rows of Table I) is therefore 90. The total is usually less than

TABLE I

ERRORS IN K AND V WHEN THE DATA WERE DERIVED FROM THREE MODELS CONTAINING WIDER RANGES OF THE TWO PARAMETERS

See text for explanation.

Solute conc.		Number of results within each error group			Median error
		<1%	1–5%	>5%	
Model (i). Water absorption rate constant					
K	s_0	22	8	48	−7.12
	s_n	15	13	50	5.79
	s_a	37	18	23	−0.31
	s_g	38	20	20	0.52
	s_e	55	13	10	0.17
	s_i	53	14	11	0.23
V	s_0	36	15	27	−1.33
	s_n	33	19	26	0.63
	s_a	55	9	14	−0.06
	s_g	56	10	12	0.03
	s_e	66	4	8	0.00
	s_i	66	3	9	0.01
Model (ii). Water absorption rate proportional to substrate concentration					
K	s_0	18	11	55	−8.40
	s_n	15	15	54	5.17
	s_a	50	6	28	−0.43
	s_g	51	7	26	0.38
	s_e	63	5	16	0.11
	s_i	64	11	9	0.18
V	s_0	33	18	33	−2.42
	s_n	32	22	30	1.14
	s_a	62	8	14	−0.09
	s_g	64	7	13	0.09
	s_e	71	5	8	0.02
	s_i	74	3	7	0.04
Model (iii). Water absorption rate proportional to substrate absorption rate					
K	s_0	5	19	57	−11.75
	s_n	6	20	55	7.36
	s_a	45	10	26	−0.53
	s_g	47	11	23	0.24
	s_e	55	11	15	0.04
	s_i	53	13	15	−0.00
V	s_0	24	20	37	−2.40
	s_n	29	17	35	1.24
	s_a	51	9	21	−0.13
	s_g	55	11	15	0.06
	s_e	63	7	11	0.00
	s_i	64	6	11	−0.02

this number, however, because for some data sets the absorption rates were high so that all the solute was absorbed before the end of the intestine was reached. In these cases K and V could not be calculated. For each model and for each algebraic function (s_0, s_n, s_a, s_g, s_e or s_i) the total number of parameter estimates are classified into three ranges according to the percentage difference between the estimated parameter and its true value. The median value of these errors was also calculated.

An attempt was made, for each data set and for each algebraic function, to correlate the percentage fall in s_0 (to s_n) with the percentage error in K. No clear patterns were apparent, because the relationship between these two variables is very dependent on the type of model used and the values of its parameters. However, it was noted that for those data sets where there was complete removal of substrate at one of the data points (i.e. with $s_0 = 4.0$ μmol/ml and $V_p = 1.0$ ml/min) then the use of s_i usually gave the least error in K. Of eleven data sets where this happened four had an error in K of less than 2% and eight had an error less than 5%. From the table and the above results it is apparent that the 'integrated mean' value (s_i) can be successfully used when water is being absorbed along with solute. In general it is a better function than the initial solute concentration (s_0), the effluent concentration (s_n), their arithmetic mean (s_a) or their geometric mean (s_g). However there is probably no significant difference between the use of my empirical function (s_e) and the 'integrated mean' value (s_i).

Since there are some, although not complete, theoretical grounds for using the 'integrated mean' value (s_i) [5] and because it is mathematically more elegant, I conclude that this is probably the preferred function to use in future for the analysis of experiments on absorption from perfused intestines.

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