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A model for steroid transport across biological membranes

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The absorption of a range of steroids (water soluble to lipid soluble) in the mouth was investigated under standard conditions and using gas chromatography. A two-compartment open model was used to describe the absorption of these steroids into, and through, the membrane. Rate constants were calculated on this basis using a feathering technique, and were used in an analogue computer program to predict steroid concentration which agreed favourably with experimental data. Correlations between absorption and partition data were made in an attempt to relate the proposed model to anatomical features of the absorptive membrane and to make comparison with models proposed to describe drug absorption across the intestinal mucosa.

Few data on the kinetic aspects of steroid transport across physiological membranes have been published. Schedl & Clifton (1961) and Schedl (1965) investigated steroid intestinal absorption in the rat and man respectively. Yotsuyanagi & Higuchi (1972), interpreting the results of Scheuplein, Blank & others (1969), reported on steroid permeation across the stratum corneum.

Current administration of steroids by both oral and sublingual routes has necessitated further investigation into the mechanisms involved and the physico-chemical aspects of steroid transport.

The oral mucosal membrane has been chosen as a typical physiological membrane. Reviews on the oral mucosal absorption of drugs have been published by Walton & Lacy (1935), Gibaldi & Kanig (1965), Beckett & Hossie (1971) and Moffat (1971).

Experimental-oral mucosal absorption measurements and determination of steroid n-heptane/buffer partition coefficients.

Apparatus. A. Pye 104, Model 84, gas chromatograph with flame ionization detector. Leeds and Northrup Speedomax G recorder, chromatograph fitted with a 4′, ¼″ o.d. glass column packed with 3% OV 17 on Chromosorb G (A.W., DMDCS treated) 80–100 US mesh. Nitrogen (carrier gas) flow rate 35 ml min^{-1}. Hydrogen pressure 9, air pressure 20 p.s.i. Detector temp. 320°. Oven temp.—see Table 1. B. Perkin-Elmer, F.11, gas chromatograph with flame ionization detector. Hitachi-Perkin-Elmer Model 159 Recorder. Chromatograph fitted with 0·5 metre, ¼″ o.d. glass column packed with 10% OV1 on Gas-Chrom Q (A.W., DMDCS treated) 100–120 US mesh nitrogen (carrier gas) flow rate 60 ml min^{-1}. Hydrogen pressure 20, air pressure 20 p.s.i. Injection port temp. 280°. Oven temp.—refer to Table 1.

Pye-Unicam SP800 ultraviolet spectrophotometer.

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Steroids. Testosterone, testosterone acetate, testosterone propionate, cortexolone and methyltestosterone were obtained as gifts from Alza Research, California, U.S.A. Oestradiol-17β, oestrone and deoxycorticosterone were obtained as gifts from Dr. M. Mitchard, University of Birmingham, Edgbaston, Birmingham, U.K.

Methandriol, oxymesterone and stanolone were obtained as gifts from Organon Laboratories, Morden, Surrey, May and Baker Ltd., Dagenham, Essex; and Lloyd-Hamol, London, W.1., U.K. respectively. Oxandrolone and norethandrolone were gifts from Mr. A. Batchelor, G. D. Searle Ltd., High Wycombe, Bucks, U.K. Progesterone, ethynyloestradiol, dehydroepiandrosterone and etiocholanolone were obtained commercially from Steraloids Ltd., Croydon, Surrey, U.K. All steroids were used without further purification. *Buffer solution.* This was McIlvaine buffer, pH 7·4 (Documenta Geigy, 6th edn).

1. Procedure for the oral mucosal absorption of steroids

Solutions of steroids were prepared at room temperature by intermittent rapid agitation (using an Ultra-Turrax homogenizer, Janke and Kunkel KG) of an excess of steroid in buffer solution for approximately 10 min, followed by filtration through Whatman no. 1 paper. Solutions of the test steroid and the internal marker were prepared similarly.

Method. One male subject (M.E.P.), aged 25, was used for all the studies since intrasubject variation has been shown to be less than intersubject variation (Beckett & Triggs, 1967).

Steroid solutions (25 ml), prepared as described, were introduced into the mouth and circulated (about 100 times per min) for a given time. Immediately after expulsion of the steroid solution, a 5 ml three second buffer rinse was used to wash out steroid remaining in the mouth. The volume and rinse time used were smaller than those described in the "General Method for Buccal Absorption" (Beckett & Triggs, 1967; Beckett and Hossie, 1971) in order to avoid undue back partitioning of steroid from the mucosa into the oral cavity.

Table 1. *Oral mucosal absorption study—analytical data for the determination of steroids in a buffer/saliva medium.*

Test steroid	Init. concn $\mu g\ ml^{-1}$	Internal marker	Solvent for extrn	System*	Column oven temp. used °C	Retention times (min) Column A (294°C) Test steroid	Column A (294°C) Internal marker	Column B (190°C) Test steroid	Column B (190°C) Internal marker
Cortexolone	21·5	Progesterone	Chloroform	A	294	5·2(1)	7·6	4·6(1)	8·3
Dehydroepi-androsterone	14·9	Oxandrolone	Ether	A	294	3·6	7·2	3·2	6·7
Deoxycorticosterone	24·0	Testosterone	Ether	A	293	14·6	5·2	— (2)	5·1
Ethynyloestradiol	4·1	Progesterone	Ether	A	290	6·1	7·6	5·8	8·3
Etiocholanolone	4·2	Stanolone	Ether	B	190	3·2	4·1	3·0	3·7
Methandriol	5·9	Progesterone	Ethyl acetate	A	285	3·6	7·6	3·8	8·3
Methyltestosterone	6·8	Progesterone	Ether	A	294	5·2	7·6	5·2	8·3
Oestradiol−17β	N.D.	Progesterone	Chloroform	B	190	5·2	7·6	4·5	8·3
Oestrone	N.D.	Progesterone	Chloroform	B	190	5·2	7·6	4·2	8·3
Oxandrolone	6·5	Dehydroepi-androsterone	Ether	A	294	7·2	3·6	6·7	3·2
Oxymesterone	N.D.	Norethandrolone	Chloroform	A	284	5·2	6·0	5·6	6·5
Progesterone	6·2	Testosterone	Ether	A	294	7·6	5·2	8·3	5·1
Stanolone	1·4	Testosterone	Ether	A	293	4·1	5·2	3·7	5·1
Testosterone	18·2	Stanolone	Ether	A	293	5·2	4·1	5·1	3·7
Testosterone acetate	N.D.	Testosterone proprionate	Ether	A	274	6·4	7·8	7·2	10·5

* See chromatographic systems.
N.D. not determined. (1) pyrolysed to 4-androstenedione. (2) 6 peaks produced.

Analysis. To each expelled steroid solution pooled with the respective rinse, 10 ml of internal marker solution (Table 1) was added and the steroids extracted with 3 × 75 ml of a suitable redistilled solvent (Table 1). After centrifugation, the pooled solvent from the 3 extractions was evaporated to approximately 2 ml on a rotary-film evaporator and transferred, washing with 2 × 2 ml of fresh solvent to a tapered evaporating tube. The solvent was evaporated to approximately 100 μl and 3 μl portions were injected onto the appropriate chromatographic column (Table 1).

2. Procedure for the determination of steroid n-heptane/buffer partition coefficients.

Steroid solutions for partition studies were prepared in aqueous buffer as described for the "Oral mucosal absorption procedure" (Section 1). For some determinations, two initial aqueous phase concentrations were used to serve as a check for: (a) association in the organic phase (see Moffat, 1968); (b) saturation of the steroid in the organic phase.

Phase composition. (i) n-Heptane saturated with McIlvaine buffer pH 7·4. (ii) McIlvaine buffer pH 7·4 saturated with n-heptane.

Method. Suitable volumes of the 2 phases (Table 2) were shaken at 37 ± 1° in a stoppered conical flask; 20 min was allowed for equilibration (see Pickup, 1973). The contents were transferred to a separating funnel and the phases allowed to separate completely (1 h at 37°).

All determinations were made in duplicate together with a control flask containing aqueous steroid solution (i.e. one phase only).

Analysis. Aqueous steroid solutions were assayed either chromatographically or spectrophotometrically (see Table 2), using peak height ratio or absorbance respectively to measure steroid concentration. The equations used were based on that of Beckett & Moffat (1969).

Table 2. *Steroid n-heptane/buffer partition coefficients at 37 ± 1°.*

Steroid	Init. concn (20°) μg ml⁻¹	Phase vol. (ml) heptane	buffer	Assay*	Partition coeff. (3 sig. figs.) (i)	(ii)	Aver.	Mean partition coeff. k hept.
Cortexolone	15·70	25	25	S	0·112	0·124	0·118	0·118
	7·85	,,	,,	S	0·126	0·110	0·118	
Dehydroepiandrosterone	10·20	5	75	C	47·8	52·4	50·1	53·5
	5·10	,,	,,	C	57·9	55·8	56·9	
Deoxycorticosterone	25·0	25	25	S	9·91	9·39	9·65	9·65
Ethynyl oestradiol	4·40	5	100	C	11·3	12·0	11·7	11·5
	2·20	,,	,,	C	10·9	11·6	11·3	
Etiocholanolone	satd	5	75	C	87·0	87·2	87·1	81·3
	50% satd	,,	,,	C	78·7	72·3	75·5	
Methandriol	satd.	10	50	C	80·5	79·7	80·1	78·8
	50% satd.	,,	,,	C	74·5	80·4	77·5	
Methyltestosterone	6·20	5	25	S	25·0	26·0	25·5	25·0
	3·10	,,	,,	S	23·6	25·3	24·5	
Oestradiol−17β	N.D.	25	25	C	4·95	4·59	4·77	4·77
Oestrone	N.D.	25	25	C	12·1	12·1	12·1	12·1
Oxandrolone	6·8	25	25	C	3·95	3·58	3·77	3·77
Oxymesterone	4·2	25	25	C	172	188	180	180
Progesterone	6·0	5	100	C	781	787	784	755
	3·0	,,	,,	C	699	752	726	
Stanolone	satd	5	75	C	158	172	165	172
	50% satd	,,	,,	C	175	182	179	
Testosterone	16·9	25	25	C	15·0	15·2	15·1	15·1
Testosterone acetate	satd	5	100	C	3150	2910	3030	3190
	50% satd	,,	,,	C	3290	3400	3350	

* C = Chromatographic. S = Spectrophotometric. N.D. not determined.

3. Determination of absolute steroid concentration in buffer solution

(a) Δ^4-3-Ketosteroids were determined using a Pye-Unicam S.P. 800 U.V. spectrophotometer using an extinction coefficient obtained from the literature. (b) Steroids other than (a) above were determined by g.l.c. assay using the appropriate solvent, internal marker and chromatograph listed in Table 1.

RESULTS

1. Oral mucosal absorption. The initial steroid concentration used for each absorption study, is presented in Table 1. The absorption data presented as semilogarithmic plots showing the percentage steroid remaining in the oral cavity, A_{corr}*, at various mucosal contact times, t_{min}, are given in Fig. 1.

Calculation of the corrected percentage unabsorbed from the oral cavity, A_{corr}

The experimentally determined (true) percentage steroid absorbed, B, is given by equation 1.

$$B = \frac{(R_1 - R_2)}{R_1} \cdot 100 \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where R_1 and R_2 are chromatographic peak height ratios determined for steroid solution before and after contact with the oral mucosa, i.e. at zero time[1] and time (t) respectively.

Equation 1 holds provided that a linear relation exists between peak height ratio and concentration for the steroids studied. Linear calibration curves (mean coefficient of variation = 4·01) were obtained (Pickup, 1973) for 8 steroids covering a wide structure range—corticosteroids, androgens and oestrogens. Linearity of detector response was assumed for the other steroids studied.

Since volume changes, due to salivary secretions, occur during absorption experiments thus altering the effective concentration in the oral cavity and these changes vary between studies, it was found necessary to adopt a small volume correction factor, fv, such that results obtained from each study could be compared.

$$fv = \left(V_1 + \frac{V_2 - V_1}{10}\right) / V_1 \quad .. \quad .. \quad .. \quad (2)$$

where V_1 and V_2 are the volumes of fluid in the mouth, initially and at time t, respectively. For all the steroids studied, V_2 did not exceed 37 ml.

Since $V_1 = 25$,

$$fv = (V_2 + 225)/250 \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The corrected percentage steroid absorbed

$$B_{corr} = B \times fv \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$A_{corr} = 100 - B_{corr} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

2. Partition coefficients

The calculated partition coefficients for each steroid are shown in Table 2. Parti-

* Defined below.

[1] Duplicate 25 ml solutions, without contact with the mouth were analysed using methods identical to those used for the expelled solutions.

tion coefficients obtained were independent of the initial steroid concentration used indicating no association in the organic phase (Moffat, 1968), and no saturation of either phase with steroid.

EXPERIMENTAL—MATHEMATICAL TREATMENT OF ABSORPTION DATA

Method and theory

The semilogarithmic plots (Fig. 1) were each in turn subjected to the technique of feathering (Doluisio, Crouthamel & others, 1970; Notari, 1971) and all indicated that

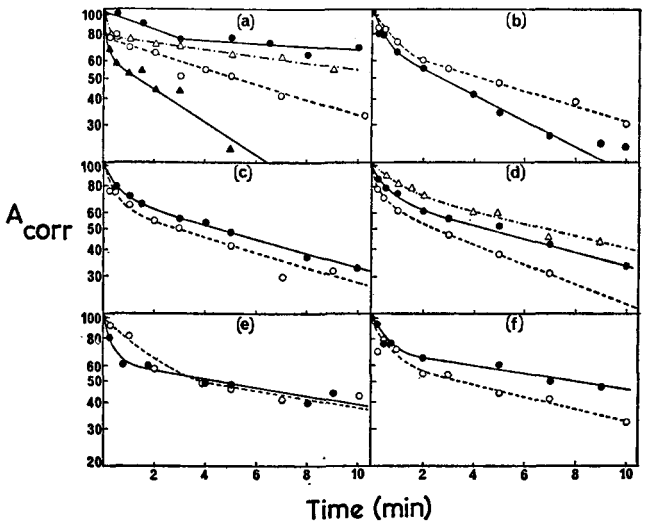

FIG. 1. The buccal absorption of steroids. Semilogarithmic plots showing the percentage steroid remaining in the oral cavity (A_{corr}) at various times t.
(a) ● cortexolone; ▲ testosterone acetate; Δ oxandrolone; ○ etiocholanolone.
(b) ● ethynyloestradiol; ○ oestrone.
(c) ● progesterone; ○ dehydroepiandrosterone.
(d) ● oxymesterone; △ testosterone; ○ stanolone.
(e) ● oestradiol-17β; ○ deoxycorticosterone.
(f) ● methyltestosterone; ○ methandriol.

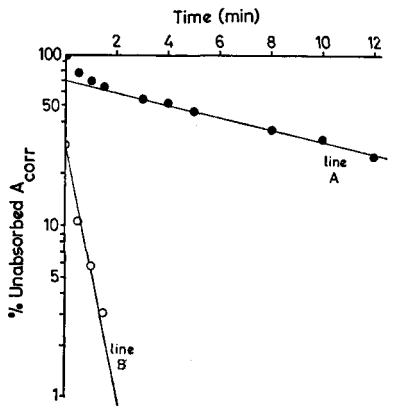

FIG. 2. Semilogarithmic plot showing: Line A drawn through points representing the percentage progesterone unabsorbed (A_{corr}) at time t min. Line B drawn through points obtained by feathering and indicating that the process can be described in terms of a biexponential process. (Notari, 1971).

the process of steroid absorption can be described in terms of a *biexponential* loss from the oral cavity (e.g. Fig. 2).

In proposing a suitable mathematical model to describe the process, the results of preliminary experiments were first considered, e.g. testosterone and progesterone were recovered from the oral mucosa by back partitioning into freshly introduced buffer solution; 33 and 14% recoveries of absorbed drug were found respectively (Pickup, 1973). A *reversible* process is thus indicated, and since incomplete recovery is evident, the process may be described as *open*—in agreement with the observed pharmacological effects after sublingual administration of steroids (Miescher & Gasche, 1942; Dunn & Hoffman, 1946; Finkler, 1947; Bickers, 1949) indicating steroid passage into general circulation. The percentage absorption of a steroid with time is similar if introduced into the oral cavity singly or as a mixture of steroids and is independent of initial concentration (Pickup, 1973), thus indicating that the absorption is a *passive* diffusional process. A simple mathematical model similar to that proposed to describe the buccal absorption of basic drugs (Beckett, Boyes & Triggs, 1968) is therefore proposed.

MODEL I

$$\underset{\text{oral cavity}}{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_2} C \quad - - ->.$$

A, B and C are mathematical compartments (Rescigno & Segre, 1966).

The constants shown in Model I were calculated in turn for each steroid studied from the semilogarithmic plots of which Fig. 2 is an example. The equations used are given by Doluisio & others (1970) and the results are shown in Table 3.

Table 3. *Calculated rate constants describing the oral mucosal absorption of steroids and using Model I, and the percentage steroid in compartment C (computer predicted and based on Model I) after 10 min oral mucosal contact time.*

Steroid	k_1 min^{-1}	k_{-1} min^{-1}	k_2 min^{-1}	Steroid	%
Cortexolone	0·057	0·049	0·055	Testosterone acetate	87
Dehydroepiandrosterone	0·721	0·940	0·192	Stanolone	62
Deoxycorticosterone	0·441	0·512	0·128	Progesterone	53
Ethynyloestradiol	0·430	0·197	0·068	Etiocholanolone	52
Etiocholanolone	0·711	1·576	0·280	Oestrone	52
Methandriol	0·508	0·675	0·169	Dehydroepiandrosterone	51
Methyltestosterone	0·818	1·902	0·216	Oxymesterone	50
Oestradiol-17β	1·053	1·338	0·091	Methandriol	46
Oestrone	0·474	0·760	0·238	Testosterone	45
Oxandrolone	1·159	3·454	0·155	Methyltestosterone	44
Oxymesterone	0·698	1·314	0·230	Deoxycorticosterone	39
Progesterone	0·540	0·932	0·249	Ethynyloestradiol	31
Stanolone	1·016	1·406	0·259	Oestradiol-17β	31
Testosterone	0·323	0·759	0·266	Oxandrolone	30
Testosterone acetate	3·130	5·473	0·610	Cortexolone	9

The following equations describe steroid transfer between compartments (assuming Model I to be a valid mathematical representation of the biological system):

$$dA/dt = -k_1 A + k_{-1} B$$
$$dB/dt = k_1 A - B(k_{-1} + k_2)$$
$$dC/dt = k_2 B$$

where A, B and C represent the percentage weight of steroid in the respective compartments at time t and k_1, k_{-1}, and k_2 are constants describing the transfer process, incorporating a volume term and having units of reciprocal time.

An analogue computer (Electronic Associates Ltd., TR-20R) linked with *X-Y* recorder (Bryans Ltd) and digital voltmeter (Roband Ltd) was used to simulate the absorptive process.

The appropriate potentiometers were adjusted to give voltage drops equivalent to the calculated constants (Table 3), and computer curves were generated for *each* steroid studied to fit the experimental data (e.g. computer fits for ethynyloestradiol and etiocholanolone are shown in Fig. 3). As illustrated in Fig. 3, computer predicted levels in compartments B and C (Model I) as a function of time—were also obtained for each steroid in turn.

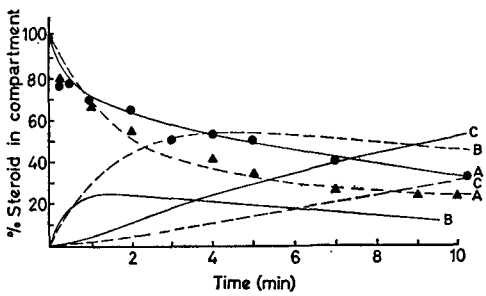

FIG. 3. Computer predicted curves and experimental data points for the oral mucosal absorption of: (a) etiocholanolone ● (b) ethynyloestradiol ▲ Lines A, B and C represent computer predicted curves for the percentage steroid in compartments A, B and C respectively at time t min. The data points are experimentally determined values (A_{corr}) for steroid in the oral cavity (compartment A by definition) at time t min.

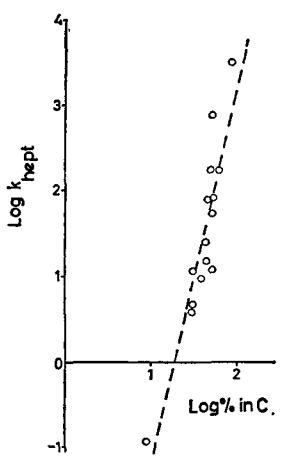

FIG. 4. The relation between the lipid character of some steroids and the computer predicted percentage steroid in compartment C (model I) after 10 min contact time. The dotted line represents line of best fit.

To demonstrate a possible relation between physico-chemical parameters and absorption data, the log of the n-heptane partition coefficient for each steroid (Table 2) was plotted against: (a) the log percentage steroid in compartment C at time t = 10 min (see Fig. 4)—this value was obtained by reading off the appropriate computer predicted curve for steroid in compartment C (e.g. see Fig. 3) and is recorded for each steroid in Table 3. (b) the log of the calculated value of k_2 (data from Table 3) (see Fig. 5).

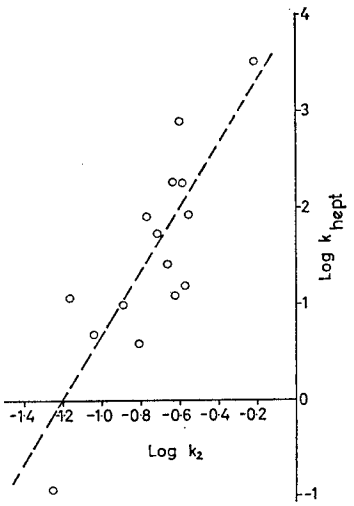

FIG. 5. The relation between the lipid character of some steroids and their calculated rate constants, k_2—The dotted line represents line of best fit.

RESULTS AND DISCUSSION OF MATHEMATICAL TREATMENT

The use of feathering as a means of obtaining the relevant rate constants is a sufficiently accurate method in the present context; the constants, when fed into the analogue computer, gave curves which fitted the original experimental data for each of the steroids studied. This is exemplified for two of steroids in Fig. 3 and supports the applicability of Model I to the biological situation.

Model I, proposed for the absorption of unionized drugs from the mouth to explain their biexponential kinetics, can be compared with models proposed to describe the intestinal absorption of drugs exhibiting similar kinetics. The two-compartment open model of Doluisio & others (1970) and the tissue compartment model of Barr & Riegelman (1970) are similar mathematically to Model I. Since epithelial tissue is common to both intestinal and oral mucosae, it is probable that a similarity exists on physical grounds also: we propose that absorption of steroids from the mouth occurs by way of partition into the surface epithelial cells (compartment B, Model I) followed by diffusion across the membrane to the blood (compartment C). The diffusion coefficient for steroid transport across the membrane would be represented by k_2 (Model I). It is possible that the rate controlling step during the diffusional process is passage across successive *lipid* cell walls and this may explain the highly significant linear logarithmic correlations obtained between the lipid character of the steroid (measured in terms of the n-heptane/buffer partition coefficient) and (a) the percentage steroid in compartment C at time t = 10 min (Fig. 4) . . . correlation coefficient r = 0·88; (b) the calculated k_2 values (Fig. 5) . . . correlation coefficient = 0·83.

In view of its similarity to established models for absorption from the gastro-

intestinal tract, we have preferred Model I to describe absorption from the mouth rather than the model proposed by Dearden & Tomlinson (1971), for the absorption of *para*-substituted acetanilides, which also describes a biexponential process. This model involves protein binding sites, the oral cavity, membrane and body fluids. Since dialysis studies (Pickup, 1973) indicate that salivary protein appears to have no kinetic influence on the absorptive process, the only possible binding site, if the model of Dearden & Tomlinson (1971) were applicable to steroid absorption, would be the membrane surface. Little evidence has been put forward to suggest that epithelial cell protein binding is rate controlling in drug absorptive processes. Secondly, a model involving protein binding is unlikely since the percentage of steroid absorbed through the oral mucosa is independent of initial concentration and is the same whether given alone or in a mixture of steroids (Pickup, 1973).

Model I is similar to that proposed for the oral mucosal absorption of basic drugs (Beckett & others, 1968) but differs from that used to describe the absorption of acids (Beckett & Moffat, 1970; Ho & Higuchi 1971; Vora, Higuchi & Ho, 1972; Wagner & Sedman, 1973). The latter could be described reasonably adequately in mono-exponential terms despite indications that in some cases biexponential treatment, as indicated by Dearden & Tomlinson (1971) might have greater relevance. It therefore seems more logical to use the *same* model (i.e. Model I) for the oral mucosal absorption of all compounds whether they be neutral, acidic or basic.

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