Table V—Point-to-Point Interpolations from Five Relationships between C and a Response Function for the Vitamin Model

Known C	Linear				Quadratic in C					
	A versus C		A' versus C		A versus log C		T versus C		T versus log C	
	C	Error, %	C	Error, %	C	Error, %	C	Error, %	C	Error, %
3	3.092	3.1	2.991	0.0	3.001	0.0	3.057	1.9	2.986	−0.5
5	5.063	1.2	5.006	0.1	5.000	0.0	4.929	−1.4	5.011	0.2
7	7.050	0.7	7.012	0.2	7.001	0.0	6.963	−0.5	7.005	0.1
9	9.042	0.5	9.015	0.2	9.001	0.0	8.796	−0.3	9.002	0.0

Graphical procedures are adequate when the assay is not of the highest quality or turbidity is measured with low precision and accuracy (±0.5% T). Computational procedures are appropriate for high quality automated assays.

SUMMARY

Common calibration lines for turbidimetric assays are more likely to be curved than straight. Fitting a best straight line to the points or even a point-to-point line causes errors in interpolated potencies. The closer the calibration line is to a straight line, the smaller will be the errors of interpolation.

Three new expressions, two for antibiotic assays and one for vitamin assays, that give straighter lines than those used previously were tested by means of three models based on real assays. The new expressions could be approximated much better by straight-line segments than could the older ones. The relationships used in official methods gave the largest computational errors. One new antibiotic equation was designed for assays employing *K. pneumoniae* as the test organism.

REFERENCES

(1) N. R. Kuzel and F. W. Kavanagh, *J. Pharm. Sci.*, **60**, 767 (1971).

(2) F. Kavanagh, *ibid.*, **60**, 1858 (1971).

(3) *Ibid.*, **64**, 844 (1975).

(4) F. Kavanagh, "Analytical Microbiology," vol. 2, Academic, New York, N.Y., 1972.

(5) H. S. Ragheb and A. M. Cummings, *J. Assoc. Offic. Anal. Chem.*, **57**, 527 (1974).

(6) N. R. Kuzel and F. W. Kavanagh, *J. Pharm. Sci.*, **60**, 764 (1971).

(7) F. W. Kavanagh, *ibid.*, **63**, 1463 (1974).

(8) F. Kavanagh and L. J. Dennin, in "Analytical Microbiology," F. Kavanagh, Ed., Academic, New York, N.Y., 1963, chap. 6.10.

(9) F. Kavanagh, "Analytical Microbiology," vol. 2, Academic, New York, N.Y., 1972, chap. 3.

(10) Code of Federal Regulations, Title 21, Part 436.106, 1974.

(11) "Official Methods of Analysis," 12th ed., Association of Official Analytical Chemists, Washington, D.C., 1975, 43.103.

(12) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975.

(13) L. Millbank, R. E. Davis, M. Rawlins, and A. H. Waters, *J. Clin. Pathol.*, **23**, 54 (1970).

(14) R. E. Davis, J. Moulton, and A. Kelly, *ibid.*, **26**, 494 (1973).

(15) E. Hoff-Jorgensen and B. Hansen, *Acta Chem. Scand.*, **9**, 562 (1955).

(16) A. H. Waters and D. L. Mollin, *J. Clin. Pathol.*, **14**, 335 (1961).

(17) W. Hewitt, "Microbiological Assay," Academic, New York, N.Y., 1977, Table 3.1.

(18) C. I. Bliss, *Bacteriol. Rev.*, **20**, 243 (1956).

(19) C. I. Bliss and C. White, in "The Vitamins," vol. VI, P. Gorgy and W. N. Pearson, Eds., Academic, New York, N.Y., 1967, p. 27.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 18, 1976, from *231 Blue Ridge Road, Indianapolis, IN 46208.*

Accepted for publication January 6, 1977.

Skin as an Active Metabolizing Barrier I: Theoretical Analysis of Topical Bioavailability

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Abstract □ A model and explicit equations were developed to be used in the experimental design and data evaluation of situations where simultaneous metabolism and transport of drugs occur in the skin. By treating the skin as a two-ply laminate composed of the stratum corneum and the viable epidermis, which contains most of the catabolic enzymes that might render a drug inactive by metabolism, equations were developed permitting the *in vitro* assessment of factors that may affect topical bioavailability *in vivo*. Two situations were investigated. In the first, the drug was placed on the dermis side of the diffusion cell and did not penetrate the stratum corneum. In the second, the drug, placed on the epidermis side, penetrated the stratum corneum and then passed through the metabolizing epidermis. Expressions for determining the metabolic rate constant from experimental data along with concentration profiles and flux expressions are given both for the drug and its metabolite.

Keyphrases □ Bioavailability, topical—model and equations developed permitting *in vitro* assessment, assuming simultaneous metabolism and drug transport in skin □ Topical bioavailability—model and equations developed permitting *in vitro* assessment, assuming simultaneous metabolism and drug transport in skin □ Metabolism, drug—simultaneous with transport in skin, model and equations developed permitting *in vitro* assessment of bioavailability □ Transport, drug—simultaneous with metabolism in skin, model and equations developed permitting *in vitro* assessment of bioavailability

In the past, the major barrier to the delivery of a topical dosage form to the site of drug action has been considered to be the stratum corneum. The concept of the epidermis as a viable, metabolizing membrane, which can provide a metabolic barrier for drug action, has long been overshadowed by the emphasis on the passive permeability properties of the most superficial skin layer. Topical bioavailability should account for not only skin permeation but also cutaneous drug metabolism.

Studies concerning the passive permeability properties of the skin as a two-ply laminate (stratum corneum and viable epidermis) showed that drugs may enter the epidermis by breaching the stratum corneum and/or by passing through skin follicles, which may be considered

essentially lipid- or water-filled pores (1–3). Beneath the stratum corneum (the major passive barrier) is the viable epidermis and the dermis. Of these two layers, the viable epidermis is metabolically the more active. Oxygen consumption was found to be 5.4 times greater in the epidermis than the dermis in the hairless mouse (4); in human skin, the activity of catechol *O*-methyltransferase was 8.3 times higher in the epidermis (5).

The purpose of the present discussion is to increase the understanding of cutaneous metabolism and transport by providing a mathematical basis for designing and evaluating appropriate experiments.

DISCUSSION

Impermeable Stratum Corneum—The preceding evidence indicates that a reasonable mathematical model for the skin might be formulated from a two-ply laminate composed of the stratum corneum and the viable epidermis. The epidermis contains most of the catabolic enzymes that render the drug inactive by metabolism, but it is not clear how the distribution of catabolic enzymes might vary with epidermal depth. Presumably, one would expect a gradual increase in metabolic activity as the basal epithelial layer is approached from the stratum corneum. In this discussion, the epidermis is considered a homogeneous membrane with respect to enzyme activity. Future studies will embody more complicated enzyme distributions and should provide an experimental basis for testing the homogeneous distribution assumption.

It may seem unusual to discuss drug bioavailability when the drug is not able to penetrate the skin. However, this unfortunate clinical situation may be utilized in certain *in vitro* experiments to yield information about the metabolic activity of the epidermis. The problem of stratum corneum permeability possibly may be altered by vehicles used in the dosage form. Such a situation was found for the permeation of vidarabine in hairless mouse skin from saline (6).

Consider the skin in a diffusion cell as illustrated in Fig. 1. In this situation, the drug is placed on the dermal side of the diffusion cell. The stratum corneum side faces the receptor compartment which contains no drug. Let the drug, A, and its major metabolite, X, be impermeable to the stratum corneum ($-s \leq x \leq -m$) but permeable to the metabolizing epidermis ($-m \leq x \leq 0$). The former condition demands that:

$$\frac{dC_A}{dx} = 0 \qquad \text{(Eq. 1)}$$

and that:

$$\frac{dC_X}{dx} = 0 \qquad \text{(Eq. 2)}$$

at $x = -m$, where C_A is the concentration of the drug and C_X is the concentration of the metabolite.

After an initial induction period[1], a steady state will be set up for the diffusing drug into the metabolizing epidermis and for the efflux of the metabolite out of the epidermis. The flux equations at $x = 0$ are:

$$F_A = -D_A \frac{dC_A}{dx} \qquad \text{(Eq. 3)}$$

$$F_A = +P_{aqA}[C_A(h) - C_A(0)] \qquad \text{(Eq. 4)}$$

$$F_X = -D_X \frac{dC_X}{dx} \qquad \text{(Eq. 5)}$$

$$F_X = +P_{aqX}[C_X(0) - C_X(h)] \qquad \text{(Eq. 6)}$$

where F_A and F_X are the steady-state fluxes for A and X, respectively; D_A and D_X are the epidermal membrane diffusion coefficients, respectively; P_{aqA} and P_{aqX} are the aqueous diffusion permeability coefficients for A and X, respectively; and $C_A(h)$ and $C_X(h)$ are the bulk phase concentrations of A and X, respectively, in the donor compartment. Flux is assumed positive when diffusion is from left to right. Within the metabolizing epidermis, it is assumed that the enzyme kinetics are first order so that:

$$D_A \frac{d^2C_A}{dx^2} - k_m C_A = 0 \qquad \text{(Eq. 7)}$$

[1] If both the drug and its metabolite are highly soluble in the epidermis, this induction period may be extended.

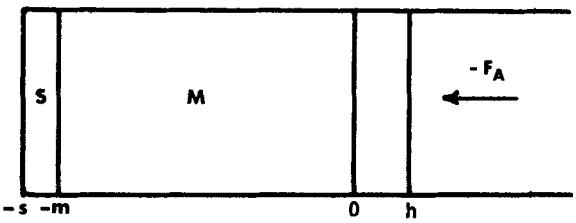

Figure 1—*Impermeable stratum corneum. Drug A moves in negative* x *direction, first penetrating the aqueous diffusion layer* ($0 \leq x \leq h$) *and then the metabolizing epidermis,* M, *located at* $-m \leq x \leq 0$. *The stratum corneum,* S, *at* $-s \leq x \leq -m$, *does not permit passage of A through it. The flux of A is considered negative, moving from right to left.*

and:

$$D_X \frac{d^2C_X}{dx^2} + k_m C_A = 0 \qquad \text{(Eq. 8)}$$

where k_m is the linear enzyme rate constant for metabolism.

Concentration Profiles for C_A *and* C_X—Initially, the drug, A, in the donor compartment diffuses from the bulk into the aqueous diffusion layer ($0 \leq x \leq h$) and penetrates the metabolizing epidermis ($-m \leq x \leq 0$). The drug concentration builds up in the epidermis gradually, consistent with the constraint that it cannot penetrate the stratum corneum ($-s \leq x \leq -m$). The product, X, gradually builds up in the epidermis and effluxes through the aqueous diffusion layer into the bulk phase. It too cannot penetrate the stratum corneum. After a sufficient time, a quasisteady state is assumed to be attained so that the concentration change of A and X at $x = 0$ and $x = h$ is sufficiently slow that Eqs. 4 and 6 are always maintained.

The solution to Eq. 7 has the general form:

$$C_A(x) = P \sinh kx + Q \cosh kx \qquad \text{(Eq. 9)}$$

where:

$$\kappa = \sqrt{k_m/D_A} \qquad \text{(Eq. 10)}$$

When the boundary conditions of Eqs. 1 and 4 are applied to Eq. 9, P and Q can be determined so that:

$$C_A(x) = \frac{\cosh \kappa(x + m)}{\cosh \kappa m + \dfrac{D_A \kappa}{P_{aqA}} \sinh \kappa m} C_A(h) \qquad \text{(Eq. 11)}$$

Integrating Eq. 8, using Eq. 11 for C_A, and evaluating the integration constants for the derivative and the function by using Eqs. 2 and 6, respectively, yield:

$$C_X(x) = C_X(h) - \frac{F_X}{P_{aqX}} \left(\frac{\kappa'}{\kappa}\right)^2 \left[\frac{\cosh \kappa m - \cosh \kappa(x + m)}{\cosh \kappa m + \dfrac{D_A \kappa}{P_{aqA}} \sinh \kappa m}\right] \qquad \text{(Eq. 12)}$$

where:

$$\kappa' = \sqrt{k_m/D_X} \qquad \text{(Eq. 13)}$$

Equations 11 and 12 give the concentration profile for $C_A(x)$ and $C_X(x)$ within the epidermal layer of the skin.

Flux Equations for A *and* X—From Eq. 3, F_A can be determined at $x = 0$. Differentiating Eq. 11 and evaluating the derivative at $x = 0$ yield:

$$F_A = -\frac{C_A(h)}{\dfrac{1}{P_{aqA}} + \dfrac{1}{D_A \kappa \tanh \kappa m}} \qquad \text{(Eq. 14)}$$

Similarly, Eq. 5 gives F_X, so using Eq. 12 yields:

$$F_X = \frac{C_A(h)}{\dfrac{1}{P_{aqA}} + \dfrac{1}{D_A \kappa \tanh \kappa m}} \qquad \text{(Eq. 15)}$$

Thus:

$$F_A + F_X = 0 \qquad \text{(Eq. 16)}$$

or the net flux of A and X is zero during quasisteady state.

Integrating Eq. 7 and using Eq. 3 yield:

$$F_A = k_m \int_0^{-m} C_A(x)\,dx \qquad \text{(Eq. 17)}$$

This equation means that the total drug flux at quasisteady state is due to the metabolism of the drug over the entire concentration profile within the epidermis.

Limits of Low and High Metabolism—Since k_m and κ are directly related by Eqs. 10 and 13, k_m or κ approaches zero in the limit of no metabolism and:

$$C_A(x) = C_A(h) \qquad \text{(Eq. 18)}$$

$$F_A = F_X = 0 \qquad \text{(Eq. 19)}$$

$$C_X(x) = C_X(h) \qquad \text{(Eq. 20)}$$

by taking the limiting values of Eqs. 11, 12, 14, and 15. Equations 18 and 20 show that, in the absence of metabolism, there is no functional dependence of the concentrations of A and X within the epidermis at steady state. Equation 19 is consistent with Eq. 17, since both equations show that there is no flux without metabolism.

In the limit of high metabolism, k_m and κ approach infinity and:

$$C_A(x) = 0 \qquad \text{(Eq. 21)}$$

$$F_A = -P_{aqA}C_A(h) \qquad \text{(Eq. 22)}$$

$$F_X = +P_{aqA}C_A(h) \qquad \text{(Eq. 23)}$$

$$C_X(x) = C_X(h) + \left(\frac{D_A}{D_X}\right)C_A(h) \qquad \text{(Eq. 24)}$$

Equation 21 shows, in the high metabolism limit, that the epidermis at $x = 0$ acts as a perfect sink for the diffusion of A through the diffusion layer.

In the aqueous diffusion layer, the transport of A is aqueous diffusion controlled (see Eq. 22), as is the transport of X, since the sum of A and X is conserved when X is not metabolized.

Calculation of κ—Equations 14 and 15 can be rearranged so that:

$$\frac{1}{\kappa} = C_1 \tanh \kappa m \qquad \text{(Eq. 25)}$$

and:

$$\frac{1}{\kappa} = C_2 \tanh \kappa m \qquad \text{(Eq. 26)}$$

respectively, where:

$$C_1 = D_A\left[\frac{C_A(h)}{-F_A} - \frac{1}{P_{aqA}}\right] \qquad \text{(Eq. 27)}$$

and:

$$C_2 = D_A\left[\frac{C_A(h)}{F_X} - \frac{1}{P_{aqA}}\right] \qquad \text{(Eq. 28)}$$

since $F_A < 0$, for diffusion of A into the epidermis:

$$C_1 = C_2 \qquad \text{(Eq. 29)}$$

if:

$$|F_A| = |F_X| \qquad \text{(Eq. 30)}$$

If Eq. 29 does not hold, then there is probably a significant amount of metabolism of X.

Equations 25 and 26 can be used to determine κ once the diffusion coefficient for A and the thickness of both the aqueous diffusion layer and the epidermis are known. Either a numerical or a graphical solution for κ can be obtained.

Permeable Stratum Corneum—Figure 2 depicts the situation of a permeable stratum corneum. The drug in the donor compartment of a diffusion cell faces the stratum corneum, while the receptor compartment faces the epidermis. The flux of the drug in Fig. 2 is in the opposite direction than in Fig. 1. This physical arrangement is analogous to the application of a topical dosage form. Metabolism is assumed to be only in the epidermis ($-m \le x \le 0$) and not in the stratum corneum ($-s \le x \le -m$). As the drug, A, moves across the stratum corneum, a flux, F_{-mA}, enters the epidermis when quasisteady state is attained. The boundary conditions at $x = -m$ are:

$$F_{-mA} = -D_A \left.\frac{dC_A}{dx}\right|_{x=-m} \qquad \text{(Eq. 31)}$$

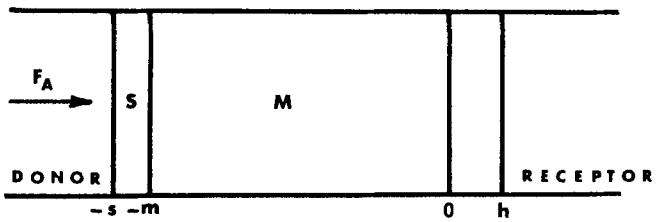

Figure 2—*Permeable stratum corneum. Drug A moves from left to right in the positive flux direction, penetrating first the stratum corneum,* S, *located at* $-s \le x \le -m$, *next the metabolizing epidermis,* M, *at* $-m \le x \le 0$, *and then the aqueous diffusion layer at* $0 \le x \le h$.

$$F_{-mA} = P_{SA}[C_A(-s) - C_A(-m)] \qquad \text{(Eq. 32)}$$

where P_{SA} is the permeability coefficient of the drug in the stratum corneum.

Within the epidermis, linear metabolism is assumed to form the major metabolite, X. At $x = 0$, both A and X diffuse out of the epidermis into the aqueous diffusion layer. The quasisteady-state fluxes of A and X are given by:

$$F_{0A} = -D_A \left.\frac{dC_A}{dx}\right|_{x=0} \qquad \text{(Eq. 33)}$$

$$F_{0A} = P_{aqA}[C_A(0) - C_A(h)] \qquad \text{(Eq. 34)}$$

$$F_{0X} = -D_X \left.\frac{dC_X}{dx}\right|_{x=0} \qquad \text{(Eq. 35)}$$

$$F_{0X} = P_{aqX}[C_X(0) - C_X(h)] \qquad \text{(Eq. 36)}$$

and the differential equations for the concentration profiles for A and X are given by Eqs. 7 and 8.

Concentration Profiles for C_A *and* C_X—The general solution for Eqs. 7 and 8 within the epidermis is given by Eq. 9. By using Eq. 9 in Eqs. 32 and 34, P and Q can be determined so that:

$$C_A(x) = P_{aqA}\frac{[P_{SA}\sinh\kappa(x+m) + D_A\kappa\cosh\kappa(x+m)]C_A(h)}{D_A\kappa(P_{SA}+P_{aqA})\cosh\kappa m + [P_{SA}P_{aqA} + (D_A\kappa)^2]\sinh\kappa m} - \frac{P_{SA}[P_{aqA}\sinh\kappa x - D_A\kappa\cosh\kappa x]C_A(-s)}{D_A\kappa(P_{SA}+P_{aqA})\cosh\kappa m + [P_{SA}P_{aqA} + (D_A\kappa)^2]\sinh\kappa m} \qquad \text{(Eq. 37)}$$

To determine $C_X(x)$, Eq. 8 is integrated twice, and Eqs. 35 and 36 are used to evaluate the constants of integration. Therefore:

$$C_X(x) = C_X(h) - F_{0X}\left[\frac{x}{D_X} - \frac{1}{P_{aqX}}\right] - \left(\frac{\kappa'}{\kappa}\right)^2[P(\sinh\kappa x - \kappa x) - Q(1-\cosh\kappa x)] \qquad \text{(Eq. 38)}$$

where:

$$P = \frac{(P_{SA}\cosh\kappa m + D_A\kappa\sinh\kappa m)P_{aqA}C_A(h) - P_{SA}P_{aqA}C_A(-s)}{D_A\kappa(P_{SA}+P_{aqA})\cosh\kappa m + [P_{SA}P_{aqA} + (D_A\kappa)^2]\sinh\kappa m} \qquad \text{(Eq. 39)}$$

and:

$$Q = \frac{D_A\kappa P_{SA}C_A(-s) + (P_{SA}\sinh\kappa m + D_A\kappa\cosh\kappa m)P_{aqA}C_A(h)}{D_A\kappa(P_{SA}+P_{aqA})\cosh\kappa m + [P_{SA}P_{aqA} + (D_A\kappa)^2]\sinh\kappa m} \qquad \text{(Eq. 40)}$$

Flux Equations for A *and* X—Application of Eqs. 33 and 31 yields:

$$F_{0A} = \frac{\operatorname{sech}\kappa m C_A(-s) - \left[1 + \frac{D_A\kappa}{P_{SA}}\tanh\kappa m\right]C_A(h)}{\frac{1}{P_{SA}} + \frac{1}{P_{aqA}} + \left[1 + \frac{(D_A\kappa)^2}{P_{SA}P_{aqA}}\right]\frac{\tanh\kappa\kappa m}{D_A\kappa}} \qquad \text{(Eq. 41)}$$

and:

$$F_{-mA} = \frac{\left[\frac{D_A\kappa}{P_{aqA}}\tanh\kappa m + 1\right]C_A(-s) - \operatorname{sech}\kappa m C_A(h)}{\frac{1}{P_{SA}} + \frac{1}{P_{aqA}} + \left[1 + \frac{(D_A\kappa)^2}{P_{SA}P_{aqA}}\right]\frac{\tanh\kappa m}{D_A\kappa}} \qquad \text{(Eq. 42)}$$

If Eq. 7 is integrated:

$$F_{-mA} - F_{0A} = k_m \int_{-m}^{0} C_A(x)\,dx \qquad \text{(Eq. 43)}$$

This equation means that the flux difference between the drug that enters the epidermis from the stratum corneum and the drug that leaves the epidermis into the aqueous diffusion layer is due to the total amount of metabolism within the epidermis. The relationship between F_{0X} and F_{-mX} can be found from Eq. 38 to be:

$$F_{-mX} - F_{0X} = \frac{\kappa'^2}{\kappa} D_X[P(\cosh(\kappa m) - 1) - Q\sinh(\kappa m)] \qquad \text{(Eq. 44)}$$

where P and Q are given by Eqs. 39 and 40, respectively. Finally, from Eq. 8:

$$F_{-mX} - F_{0X} = F_{0A} - F_{-mA} \qquad \text{(Eq. 45)}$$

F_{-mX} arises because the stratum corneum is permeable to the metabolite, X, so that X diffuses back into the donor chamber.

Limits of High and Low Metabolism—In the limit of no metabolism when κ tends to zero, Eqs. 37, 38, 41, and 42 become:

$$C_A(x) = \frac{\left[\frac{1}{D_A/(m+x)} + \frac{1}{P_{SA}}\right]C_A(h) - \left[\frac{1}{D_A/x} - \frac{1}{P_{aqA}}\right]C_A(-s)}{\frac{1}{P_{aqA}} + \frac{1}{P_{SA}} + \frac{1}{D_A/m}} \qquad \text{(Eq. 46)}$$

$$C_X(x) = C_X(h) \qquad \text{(Eq. 47)}$$

$$F_{0A} = F_{-mA} \qquad \text{(Eq. 48)}$$

$$F_{0A} = \frac{C_A(-s) - C_A(h)}{\frac{1}{P_{aqA}} + \frac{1}{P_{SA}} + \frac{1}{D_A/m}} \qquad \text{(Eq. 49)}$$

Conversely, in the limit of high metabolism:

$$C_A(s) = 0 \qquad \text{(Eq. 50)}$$

$$C_X(x) = C_X(h) + F_{0X}\left[\frac{1}{P_{aqA}} - \frac{x}{D_X}\right] \qquad \text{(Eq. 51)}$$

$$F_{0A} = -P_{aq}C_A(h) \qquad \text{(Eq. 52)}$$

$$F_{-mA} = P_{SA}C_A(-s) \qquad \text{(Eq. 53)}$$

REFERENCES

(1) R. J. Scheuplein, *J. Invest. Dermatol.*, **45**, 334 (1965).

(2) *Ibid.*, **48**, 79 (1967).

(3) R. J. Scheuplein, I. H. Blank, G. J. Brauner, and D. J. MacFarlane, *J. Invest. Dermatol.*, **52**, 63 (1969).

(4) O. D. Laerum, *ibid.*, **52**, 204 (1969).

(5) J. Bamshad, *ibid.*, **52**, 351 (1969).

(6) H. Y. Ando, N. F. H. Ho, and W. I. Higuchi, *J. Pharm. Sci.*, **66**, 755 (1977).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 18, 1975, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.*

Accepted for publication January 14, 1977.

Appreciation is expressed to Mr. J. L. Fox for assistance on the mathematical aspects of diffusion and to Ms. Aurora Calvo for editing and proofreading the manuscript.

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pH-Stat Titration of Aluminum Hydroxide Gel

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Abstract □ The pH-stat titration of aluminum hydroxide gel was evaluated and was affected by pH, temperature, concentration, and ionic strength. Control of these parameters resulted in a highly sensitive and reproducible *in vitro* antacid test. The utility of the pH-stat test was illustrated by monitoring the aging of several carbonate-containing aluminum hydroxide gels and by comparing the antacid properties as measured by the pH-stat titration, the acid-consuming capacity, the Rossett–Rice test, and the test proposed by the Food and Drug Administration Drug Evaluation Panel. The pH-stat titration also was useful for relatively nonreactive aluminum hydroxide gels. The use of sodium fluoride as the reaction medium extended the capability of the pH-stat titration to monitor the aging of chloride-containing gels. The pH-stat titrigram was interpreted in terms of a previously published polymer model of the structure of a chloride-containing aluminum hydroxide gel. The acid reactivity of relatively nonreactive gels is believed to be due totally to the chemical neutralization of acid, because the milliequivalents of aluminum ion appearing in solution is the same as the milliequivalents of acid neutralized throughout the pH-stat titration.

Keyphrases □ Aluminum hydroxide gel—pH-stat titration evaluated, aging monitored, compared to other tests □ pH-stat titration—evaluated for aluminum hydroxide gel, aging monitored, compared to other tests □ Gels—aluminum hydroxide, pH-stat titration evaluated, aging monitored, compared to other tests □ Aging—aluminum hydroxide gel, monitored with pH-stat titration, compared to other tests □ Antacids—aluminum hydroxide gel, pH-stat titration evaluated, aging monitored, compared to other tests

Available *in vitro* antacid tests may be broadly classified as static or dynamic. The acid-consuming capacity test (1), the most widely used static test, determines the total amount of acid neutralized during 1 hr at 37°. The dynamic methods may measure the pH profile during acid neutralization or determine the acid neutralization rate at a constant pH. The test of Holbert *et al.* (2) and the Rossett–Rice test (3) attempt to simulate the stomach and record the pH profile during acid neutralization. Brody and Bachrach (4) first proposed a test that measures the acid neutralization rate at a constant pH. This test subsequently was automated (5) and recently was correlated with *in vivo* acid neutralization by aluminum hydroxide gel (6).

The Food and Drug Administration (FDA) Drug Evaluation Panel on antacids proposed a test (7) that combines the static approach with the dynamic pH profile.

A sensitive *in vitro* antacid test was needed to study the structure and mechanism of neutralization of aluminum hydroxide gel and to optimize the production of aluminum