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Report

Percutaneous Absorption of Benzoic Acid Across Human Skin. II. Prediction of an *in Vivo*, Skin-Flap System Using *in Vitro* Parameters

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The possibility of predicting the behavior of *in vivo* systems based on physical and chemical parameters determined by *in vitro* experiments is examined using benzoic acid. The physical and chemical parameters governing percutaneous absorption of benzoic acid—permeability, partition coefficient, and skin thickness—were determined by *in vitro* experiments as described in Ref. 1. These parameters were used, in combination with benzoic acid elimination kinetics, to predict the results of *in vivo* experiments using a comprehensive mathematical model. The *in vivo* system consists of a congenitally athymic (nude) rat with a surgically constructed human skin sandwich (HSSF) flap on which a donor cell is placed. To apply the *in vitro* parameters to an *in vivo* system requires a suitable pharmacokinetic model describing distribution and elimination for benzoic acid in the nude rat. Blood concentrations of benzoic acid following a bolus intravenous injection are closely described by a two-compartment open pharmacokinetic model with elimination occurring from only one compartment. The mathematical model of the rat-donor cell system combines this two-compartment model of the rat with a percutaneous absorption model to provide useful estimates of the measured *in vivo* blood levels. Comparisons of predicted and measured results suggest that the parameters determined by *in vitro* experimentation can be used to predict the behavior of complex *in vivo* systems, if a suitable mathematical model is available.

KEY WORDS: percutaneous absorption; mathematical modeling; human skin sandwich flap; *in vivo*.

INTRODUCTION

Percutaneous drug delivery is an important technique for the treatment of cutaneous and systemic disorders. Drugs applied to the skin are often used in a variety of preparations which have not always been evaluated for their relative efficacy, that is, the location and amount of drug they provide to the skin or target system. Clinical studies to evaluate these differences are costly and laborious. Hence, simple *in vitro* methods of assessing the effectiveness of preparations are an attractive alternative, if the conditions under which *in vitro* results are extendable to *in vivo* systems can be defined.

This study examines the applicability of *in vitro* parameters to *in vivo* systems through a comprehensive mathematical model developed in the Appendix. Experimental procedures and data analysis for the *in vitro* experiments are discussed in Ref. 1. The *in vivo* system consists of a congenitally nude rat with an isolated human skin sandwich flap on which a donor cell containing a benzoic acid solution is placed. This arrangement allows measurement of benzoic acid concentrations in the flap (C_F) and systemic blood (C_S) as a function of time. The skin sandwich flap provides a unique opportunity for separating systemic and local effects. A schematic of the *in vivo* system is given in Fig. 1. The key components of the *in vivo* system include the donor cell, the skin barrier, the sandwich flap, and the two-compartment model of the rat. Because of the flap, the rat is actually represented by three compartments.

The key parameters determined by *in vitro* experiments include the partition coefficients K_m and K_F (flow cell fluid to skin and blood to skin, respectively), the stratum corneum barrier effective diffusion coefficient D, and the hydrated thickness of the stratum corneum h. The partition coefficient from blood to skin was estimated using a saline-skin system in Ref. 1. According to Ref. 1, the epidermis and dermis do not present a significant barrier to the movement of benzoic acid through the skin, relative to the stratum corneum. Additional *in vivo* experiments are required to determine the pharmacokinetic model for benzoic acid elimination in the nude rat.

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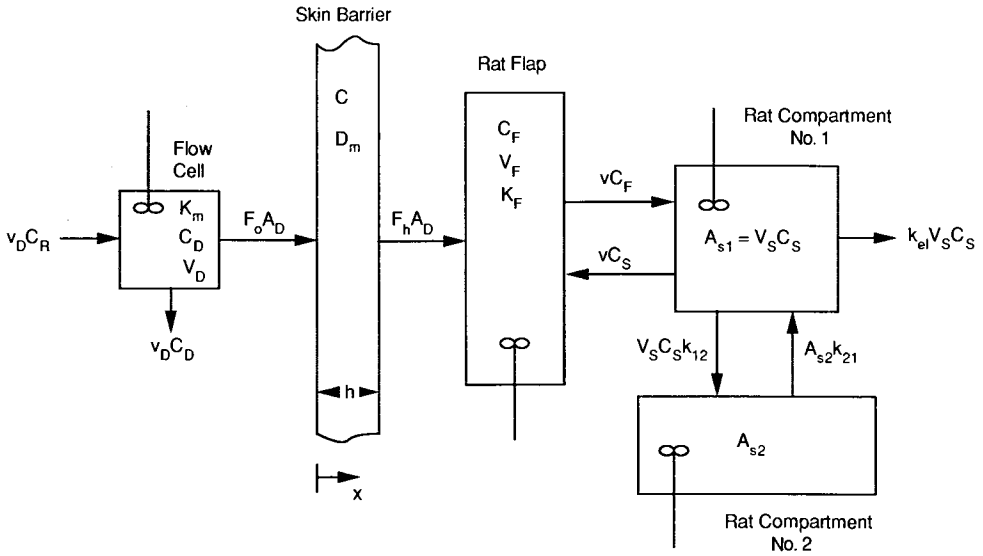

Fig. 1. Schematic of the flow cell/human skin sandwich flap system.

MATERIALS AND METHODS

Chemicals and Solutions

Solutions of radioactive benzoic acid in distilled water were prepared from radiolabeled (7-^{14}C) and nonradiolabeled acid. The radiolabeled material was obtained from New England Nuclear with a specific activity of 0.0876 μCi/μg. Nonradiolabeled benzoic acid was used as purchased from Sigma (St. Louis, MO). Cyclosporin A (Sandimmune) was purchased from Sandoz (East Hanover, NJ).

Human Skin Sandwich Flap

The human skin sandwich flap is constructed with fresh dermatomed (0.5-mm) human abdominal skin and congenitally athymic rats in three stages which are identical to the procedure outlined in Refs. 2 and 3 for rat skin sandwich flaps, with the addition of cyclosporin therapy to prevent rejection of the graft. The result is an isolated flap, with viable human skin on one side and host rat skin on the other, which is supplied by a single accessible artery and drained by a single accessible vein. Concentrations of the permeating substance in the blood leading to and from the flap can be measured directly by sampling the contralateral leg vein and the vein immediately draining the skin flap, respectively.

Flow Cell

Benzoic acid solution was continuously supplied to the skin flap using a stirred, water-jacketed cell. A flow rate of 1 ml/hr to the cell was sufficient to prevent a significant reduction in the acid concentration in the donor cell chamber. The cell is shown schematically in Fig. 2 and its relation to the entire *in vivo* system is shown in Fig. 1. The cell is constructed of glass and its contents are stirred remotely by a magnetic stir bar which is held by a rotor supported by a Teflon frame. The drug chamber volume is approximately 1 ml and the diameter of skin surface exposed to the solution is 1.0 cm. The flow cell is attached to the skin using a flat ring of silicon adhesive (3 mm thick) which was made by Thomas Petelenz of the Center for Engineering Design, University of Utah. The water supplied to the jacket is maintained at 35°C.

In Vivo Percutaneous Absorption Experiments

The rats were anesthetized intraperitoneally (100 mg/kg) with Ketachlor (Parke-Davis, Morris Plains, NY) and maintenance doses (33 mg/kg) were given every 45 min. The rats were kept warm during the experiments with a small heating pad and their core temperatures were continuously monitored. The effect of the anesthetic on skin temperature was not determined. The flow cell was attached to the human skin surface of the sandwich flap. The solution of ^{14}C-benzoic acid in distilled water (2 mg/ml; sp act, 2.46×10^{-5} μCi/μg) was supplied to the flow cell continuously at the rate of 1 ml/hr using a syringe pump.

Flap and systemic blood samples were collected every 30 min, from the vein immediately draining the skin flap and the contralateral leg vein, respectively, over a 4-hr experiment. The amount of benzoic acid in the blood samples was determined by scintillation counting (Beckman LS 8100 scintillation counter, Irvine CA). Blood flow rates in the HSSF

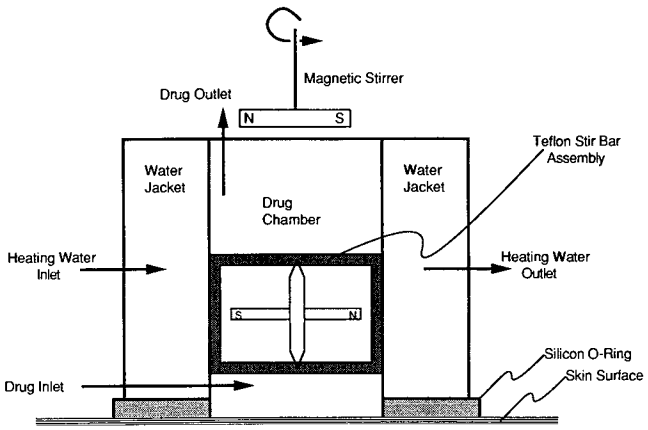

Fig. 2. Schematic of the flow cell used for delivering solution to the skin surface.

were estimated as outlined by Ref. 4 using laser Doppler velocimetry (LDV). The anesthetic decreased cutaneous blood flow rates slightly.

Elimination Studies

Elimination studies were performed on congenitally nude rats without skin sandwich flaps. Concentrations of benzoic acid in the blood were monitored over a 24-hr period following the bolus, intravenous injection of 0.6 ml of ^{14}C-benzoic acid (0.42 mg/ml; sp act, 0.16 μCi/μg) in saline solution. Concentrations were determined by scintillation counting. In determining the concentrations, it was assumed that the concentration of the benzoic acid derivative, hippuric acid, was relatively low due to its high excretion rate. The rats were anesthetized as described above during the first 4 hr of the experiment to match the *in vivo* experimental conditions.

RESULTS AND DISCUSSION

The primary goal of this study is to show that physical and chemical parameters obtained from *in vitro* experiments can be used, in conjunction with mathematical models of *in vivo* systems, to predict the behavior of *in vivo* systems. A mathematical model of the rat-flap system is presented in the Appendix. The key parameters and their values are listed in Table I. Several of the parameters listed in Table I can be obtained only by studying *in vivo* systems, e.g., pharmacokinetic parameters and blood flow rates. These include the first-order rate constants k_{21}, k_{12}, and k_{el}; the volumes of distribution V_S and F_F; and the blood flow rate v. All of these quantities vary considerably between individual animals. They may also vary during an experiment. The blood flow rate shows considerable variation for the same rat during an experiment, based on laser-Doppler velocimetry (LDV) measurements. The mathematical model was used to estimate the sensitivity of benzoic acid concentrations to all uncertain parameters. In particular, the blood flow rate has a significant impact on concentrations in the flap. All of the concentrations and the flux are insensitive to the flap volume, V_F. The following mathematical modeling calculations assume a constant blood flow rate.

Predicted and measured concentrations of benzoic acid in the systemic blood are compared in Fig. 3. The figure shows the range in the predicted values based on the standard deviations of the parameters in Table I. The range in the predictions was obtained by increasing or decreasing all of the parameters by one standard deviation in such a way as to produce the largest possible deviations above and below the mean. Uncertainties in the skin thickness and the pharmacokinetic parameters are primarily responsible for the range in the predictions. The predicted effect of blood flow rate v is negligible. This weak dependence is reasonable given that decreasing blood flow rate increases concentration in the flap blood, which feeds into the systemic compartment. That is, the decreased flow from the flap is compensated for by the increased concentration in the flap compartment.

The predicted systemic concentrations are somewhat higher than the measured values. This difference may indicate that physical and chemical parameters determined *in vitro* are slightly different from those that describe an *in vivo* system. Experiments performed with a saline/water system in Ref. 1 indicate that K_F may be a factor of 2 less than K_m. The model predicts that changes in K_F of this magnitude have no effect on concentrations of benzoic acid in the rat. However, there is considerable uncertainty in the pharmacokinetic parameters which are determined from *in vivo* experiments under conditions which are not identical to the absorption experiments. That is, the absorption experiments involve a gradual delivery of the acid, while the pharmacokinetic studies utilize a bolus injection. The pharmacokinetic response to these two rates of administration may be quite different. Benzoic acid is excreted mainly as hippuric acid. The synthesis of hippuric acid depends on the enzyme systems of the liver and on glycine availability in the liver. It is conceivable that bolus delivery depletes glycine stores, whereas a gradual delivery might not. This could account for the predicted systemic values being high.

Predicted and measured concentrations of benzoic acid

Table I. Key Parameters and Their Values

Parameter or constant	Value
A_D	0.785 cm^2
h	0.003 ± 0.001 cm
C_R	1490. μg/ml
D_m	2.1E-5 ± 0.3E-5 cm^2/hr
k_{12}	2.4 ± 1. hr^{-1}
k_{21}	0.388 ± .078 hr^{-1}
K_m	4.8 ± .1
k_{el}	2.3 ± 1.3 hr^{-1}
K_F	4.8 ± .1
N	25
v	60 ml/hr
v_D	1. ml/hr
V_D	1. ml
V_F	0.736 ml
V_s	74 ± 25 ml

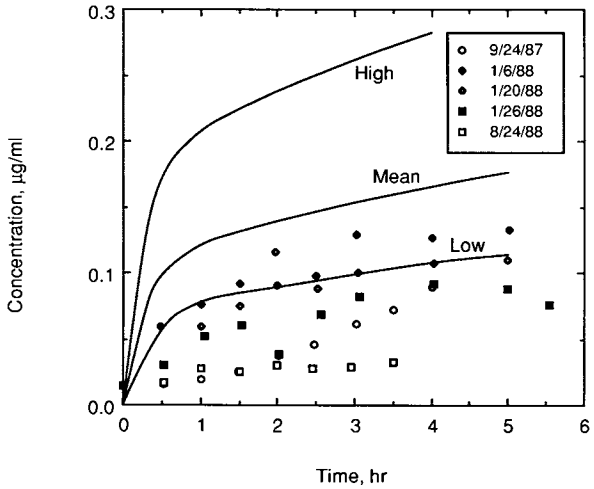

Fig. 3. Concentrations of benzoic acid in the systemic blood as measured in five different rats. The high and low predictions show the range in the predicted values based on the parameters in Table I.

in the flap blood are shown in Fig. 4. The figure shows the range in the predicted values based on the uncertainties in the parameters in Table I. The range in these predictions does not include the effects of uncertainty in the blood flow rate.

The predicted sensitivity of concentrations of benzoic acid in the flap to blood flow rate is shown in Fig. 5. Decreasing blood flow rate from 1 to 0.25 ml/hr increases flap concentrations by a factor of 3.

Predicted and measured flux levels are shown in Fig. 6. The figure shows the range in the predicted values based on the uncertainties in the parameters in Table I. Under the conditions examined, the flux rate is virtually unaffected by blood flow rate. The predicted fluxes are insensitive to blood flow rate because the concentration gradient in the barrier does not change significantly for the range of flow rates examined. The high measured flux values (1/6/88) may be due to high blood flow readings (measured using LDV) during periods of actual low flow. If this condition occurred, then Eq. (13), with $dC_F/dt = 0$, shows that calculated fluxes would be high:

$$F_h = \frac{v(C_F - C_S)}{A} \quad (1)$$

This problem is compounded by the high concentrations that occur in the flap blood during periods of low blood flow rate. The measured blood flow rate for 1/6/88 is 1.7 ± 0.2 ml/min. In light of Fig. 5, the measured value may be high since the predictions in the vicinity of the 1/6/88 data are for a flow rate of 0.25 ml/min. Equation (1) was used to estimate the measured flux levels shown in Fig. 6 from measured values of v, C_F, and C_S.

Finally, because benzoic acid has a relatively high effective diffusion coefficient in human skin, the rate of flux rapidly reaches the steady-state value given by Eq. (23) or (24). The predicted fluxes shown in Fig. 6, for times greater that about 20 min, can be readily obtained directly from the integrated form of Eq. (23) and the necessary *in vitro* parameters:

$$\text{flux} = \frac{D_m K_F C_D}{h}$$

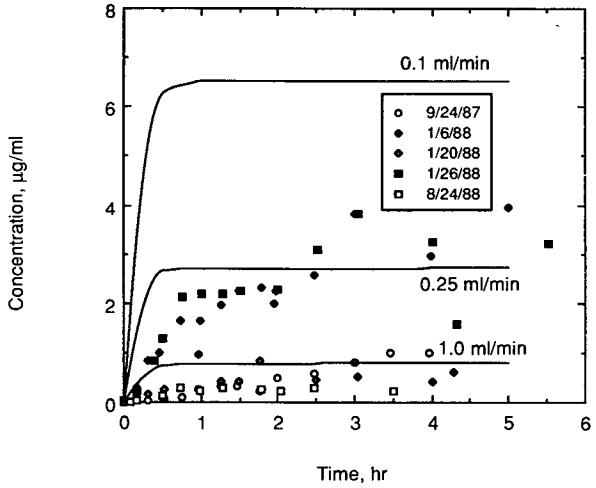

Fig. 5. Predicted (solid lines) effects of blood flow rate to the flap (v) on benzoic acid concentrations in the flap blood. The systemic concentrations and the flux are insensitive to blood flow rate.

CONCLUSIONS

In vitro experiments provide model parameters which can be used to predict *in vivo* results provided that the pharmacokinetics of the substance being considered are known. In general, the modeling assumption of constant blood flow rate provides useful, semiquantitative estimates against which the *in vivo* results can be compared. These conclusions demonstrate the possibility of using simple *in vitro* tests to evaluate the effectiveness of topical drugs without having to perform extensive clinical studies, provided that all of the parameters required to do so are available.

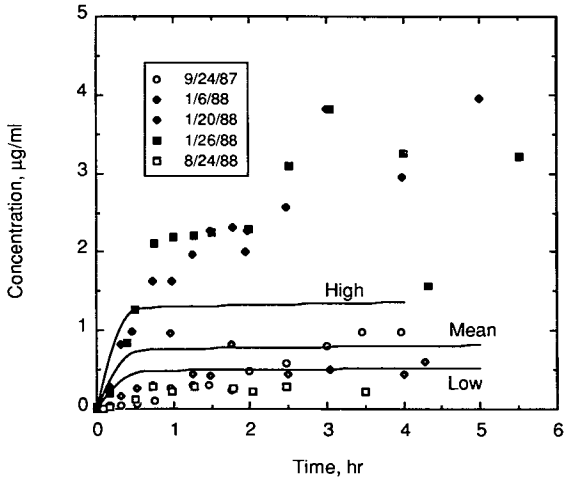

Fig. 4. Concentrations of benzoic acid in the flap blood measured in five different rats. The high and low predictions indicate the predicted range in concentrations for the parameters summarized in Table I.

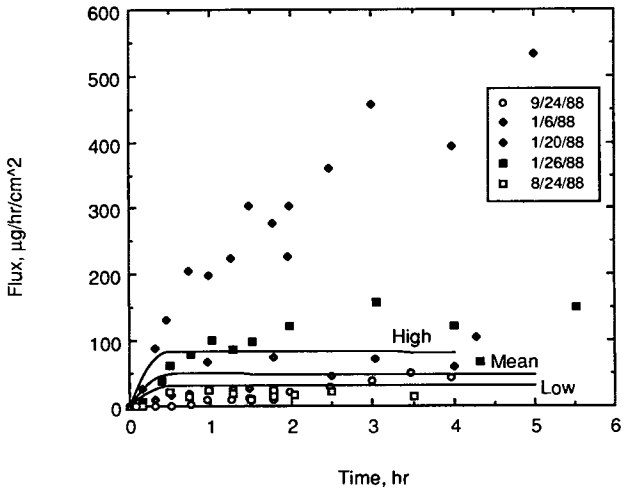

Fig. 6. Predicted and measured fluxes of benzoic acid into the flap. The high and low predictions show the range in predicted fluxes based on the parameter values in Table I.

APPENDIX: THEORY

A mathematical model of the flow cell/human skin sandwich flap system described above is developed and solved in this appendix. All parameters used in the model were obtained from the *in vitro* and *in vivo* experiments described above.

A schematic of the flow cell/human skin sandwich flap system is shown in Fig. 1. The system consists of four compartments and a permeable skin barrier. The flow cell compartment delivers drug to the skin surface. The drug partitions (K_m) from the flow cell solution to the skin surface and then diffuses through the skin. At the skin/flap interface the drug partitions (K_F) from the skin to the rat flap tissues and is carried by the blood to rat compartment number 1. Elimination occurs only from compartment 1. Rat compartment 2 serves as a drug reservoir. All four compartments in Fig. 1 are assumed to be completely stirred.

Elimination and Distribution of Benzoic Acid in the Rat

The linear, two-compartment open model used to represent the rat is shown schematically in Fig. 7. This particular model was selected based on benzoic acid elimination studies which were performed on nude rats. From Ref. 5, the equations for the model are

$$C_s = Ae^{-\alpha t} + Be^{-\beta t} \tag{2}$$

where

$$V_S = \frac{\delta}{A + B} \tag{3}$$

$$k_{12} = \frac{A\beta + B\alpha}{A + B} \tag{4}$$

$$k_{el} = \frac{\alpha\beta}{k_{21}} \tag{5}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{el} \tag{6}$$

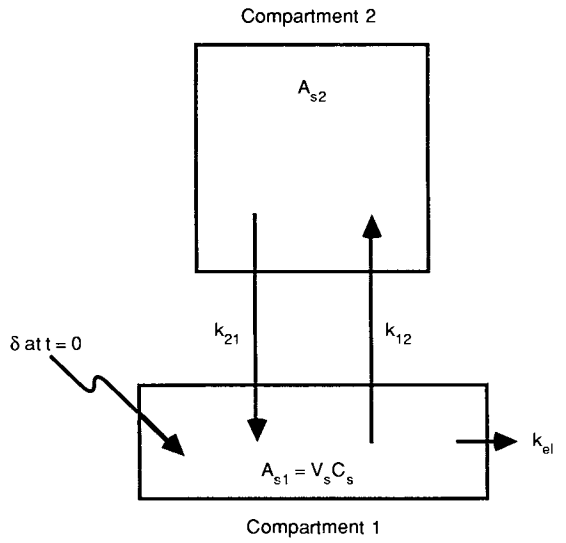

Fig. 7. Schematic of the linear, two-compartment open model used to describe the distribution and elimination of benzoic acid in a rat. The dose δ is bolus and intravenous.

The constants A, B, α, and β can be obtained by fitting Eq. (2) to elimination data, where the dose δ is administered by bolus, intravenous injection. Typical elimination data for benzoic acid blood concentrations are shown in Fig. 8. The solid line is obtained by fitting Eq. (2) to the data. Best estimates for the parameters A, B, α, and β were obtained by nonlinear least-squares regression after Ref. 6. Table I summarizes the parameters calculated from Eqs. (3) to (6) for three different rats. Note that a volume for rat compartment number 2 is not necessary for the model.

Governing Equations for the Flow Cell/Flap System

The governing equations for the flow cell/flap system are obtained by performing material balances on benzoic acid for each compartment and the skin. The development which follows is based on the following assumptions.

1. The flow cell, flap, and rat compartments are completely stirred.
2. The benzoic acid is present at low concentrations so that its presence or absence has negligible effect on the flow rates v and v_D.
3. The effective diffusion coefficient D_m is a constant.
4. The partitioning of benzoic acid into or out of the skin is instantaneous.
5. The rat flap volume F_F is roughly 1% of the volume of compartment number 1. This assumption is based on an observed flap-to-rat weight ratio of about 0.01.
6. There is no binding or metabolism of benzoic acid in the skin.
7. The principle barrier to diffusion is the stratum corneum.
8. The blood flow rate (v) is constant.
9. The benzoic acid derivative, hippuric acid, is excreted rapidly relative to benzoic acid such that its concentration in the blood remains relatively low.

A benzoic acid material balance on the flow cell gives

$$V_D \frac{dC_D}{dt} = v_D C_R - v_D C_D - F_O A_D \tag{7}$$

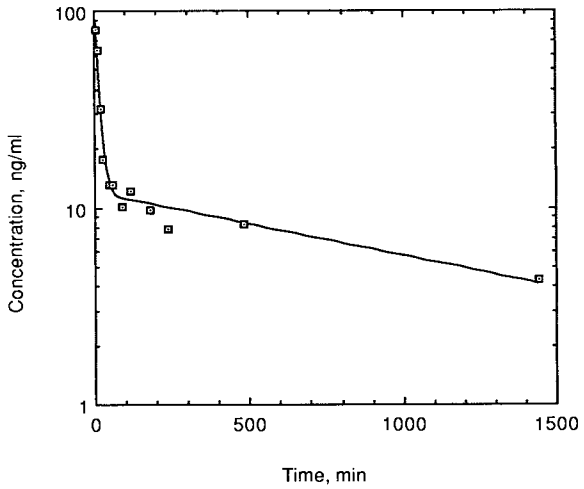

Fig. 8. Benzoic acid concentrations in the blood following bolus, intravenous injection. The solid line is a fit of Eq. (2) to the data.

where

$$\text{at } t = 0, \quad C_D = C_R \tag{8}$$

A mass balance over an element of skin gives the partial differential equation

$$\frac{\partial C}{\partial t} = D_m \frac{\partial^2 C}{\partial x^2} \tag{9}$$

where

$$\text{at } t = 0, \quad C(x,0) = 0 \tag{10}$$

$$\text{at } x = 0, \quad C(0,t) = C_D K_m \tag{11}$$

$$\text{at } x = h, \quad C(h,t) = C_F K_F \tag{12}$$

The boundary conditions (10) and (11) are based on assumption (4). A material balance on the flap yields

$$V_F \frac{dC_F}{dt} = vC_s + F_h A_D - vC_F \tag{13}$$

where

$$\text{at } t = 0, \quad C_F = 0 \tag{14}$$

For rat compartment number 1 the governing equation is

$$V_s \frac{dC_s}{dt} = vC_F + A_{s2}k_{21} - vC_s - V_s C_s(k_{12} - k_{el}) \tag{15}$$

where

$$\text{at } t = 0, \quad C_s = 0 \tag{16}$$

Finally, for compartment number 2 the governing equation is

$$\frac{dA_{s2}}{dt} = V_s k_{12} C_s - k_{21} A_{s2} \tag{17}$$

where

$$\text{at } t = 0, \quad A_{s2} = 0 \tag{18}$$

Solution

The solution to Eqs. (7) through (18) proceeds as follows. The space derivative of Eq. (9) is approximated by finite differencing

$$\frac{\partial^2 C}{\partial x^2} = \frac{C_{i-1} - 2C_i + C_{i+1}}{(\Delta x)^2} \tag{19}$$

(19)

where

$$\Delta x = h/(N - 1)$$

If the skin barrier is divided by N nodes, $N - 2$ ordinary differential equations are used to approximate Eq. (9):

$$\frac{dC_i}{dt} = D\left[\frac{C_{i-1} - 2C_i + C_{i+1}}{(\Delta x)^2}\right], \quad i = 2, \quad N - 1 \tag{20}$$

and at the skin boundaries,

$$C_1 = K_m C_D \tag{21}$$

$$C_N = K_F C_F \tag{22}$$

Hence, the system of Eqs. (7) through (18) are reduced to $N + 2$ equations which can be integrated in parallel. The integration is accomplished by a fourth-order Runge–Kutta method with adaptive step size control (6).

Approximations for the fluxes F_o and F_h in Eqs. (7) and (13) can be obtained by Taylor-series expansions:

$$F_0 = -D_m\left(\frac{\partial C}{\partial x}\right)_{x=0} = -D_m\left[\frac{-3C_1 + 4C_2 - C_3}{2\Delta x}\right] \tag{23}$$

$$F_h = -D_m\left(\frac{\partial C}{\partial x}\right)_{x=h} = -D_m\left[\frac{C_{N-2} - 4C_{N-1} + 3C_N}{2\Delta x}\right] \tag{24}$$

The differencing formulas in Eqs. (20), (23), and (24) are all second-order accurate in x.

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NOTATION

A_D	Contact area between flow cell fluid and skin, cm^2
A	Preexponential factor in Eq. (1), μg/ml
A_{s1}	The total amount of drug in rat compartment number 1, μg
A_{s2}	The total amount of drug in rat compartment number 2, μg
h	Thickness of skin barrier, cm
B	Preexponential factor in Eq. (1); μg/ml
C	Drug concentration in the skin, μg/ml
C_D	Drug concentration in the flow cell, μg/ml
C_F	Drug concentration in the flap blood, μg/ml
C_R	Drug concentration in the drug reservoir supplying the flow cell, μg/ml
C_S	Drug concentration in the systemic or rat compartment number 1 blood, μg/ml
D_m	Effective diffusion coefficient for drug in skin, cm^2/hr
Δx	The spacing between nodes in the skin, cm
F_h	Flux of drug out of skin at $x = h$, $\mu g/cm^2 \cdot hr$
F_o	Flux of drug into skin at $x = 0$, $\mu g/cm^2 \cdot hr$
k_{12}	First-order rate constant for transfer of drug from rat compartment number 1 to rat compartment number 2, hr^{-1}
k_{21}	First-order rate constant for transfer of drug from rat compartment number 2 to rat compartment number 1, hr^{-1}
K_D	Partition coefficient from the flow cell fluid to the skin at $x = 0$
k_{el}	First-order rate constant for elimination of acid from rat compartment number 1, hr^{-1}

K_F Partition coefficient between the flap blood and the skin at $x = h$
N The number of nodes in the skin barrier
t Time, hr
v Volumetric blood flow rate between the rat flap and the central rat compartment, ml/hr
v_D Volumetric flow rate into and out of flow cell, ml/hr
V_D Flow cell volume, ml
V_F Rat flap volume, ml
V_S Volume of rat compartment number 1, ml
x Distance from skin surface, cm

Greek Symbols

α Time constant in Eq. (1), hr^{-1}
β Time constant in Eq. (1), hr^{-1}
δ Dose, μg

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