*Pharmaceutical Research, Vol. 5, No. 9, 1988*

*Research Article*

# Solute Absorption from the Airways of the Isolated Rat Lung. I. The Use of Absorption Data to Quantify Drug Dissolution or Release in the Respiratory Tract

**Ralph W. Niven[1] and Peter R. Byron[2,3]**

*Received December 28, 1987; accepted March 17, 1988*

Coprecipitates of fluorescein and magnesium hydroxide demonstrate delayed absorption relative to fluorescein solutions when administered to the airways of the isolated perfused rat lung (IPRL). Perfusate concentration vs time profiles showed that dissolution and not epithelial permeability was the rate-controlling factor in the airway-to-perfusate transfer process. A simple data deconvolution method was developed to determine the fluorescein release from the microparticulate coprecipitates in the airways. The deconvolution technique is generally applicable and provides values for undissolved solute remaining in the airways as a function of time provided that (a) significant binding and/or metabolism does not occur, (b) absorption from solution is apparent first order, and (c) all solid or dissolved material reaching perfused regions is absorbed within the lifetime of the preparation. Increased release rates of fluorescein occurred from precipitates containing greater starting concentrations of the dye. Dissolution profiles were similar to those that occur for log-normally distributed powders. The analysis of two unusual time profiles implied that the regional distribution of solid and dissolved material, between perfused areas and nonperfused areas, could be nonhomogeneous despite the use of a standardized dosing technique. The studies describe a method of using the IPRL with the potential to screen aerosol formulations for extended dissolution in the respiratory tract.

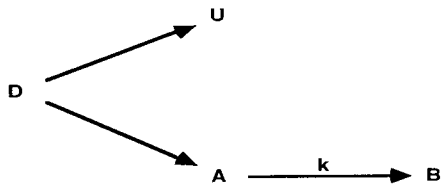
KEY WORDS: aerosol; absorption; deconvolution; dissolution; fluorescein; isolated lung.

## INTRODUCTION

Chemical compounds have been administered to the lungs of different species in many ways (1–9). When absorption is the purpose of the study, administration to the respiratory tracts of intact whole animals may produce different results for solute transfer kinetics dependent upon the sites of deposition in the airways (1,2,4,6,9). This is due, at least in part, to the existence of a complete bronchial, as distinct from pulmonary, circulation but is not the case in isolated lungs, where absorption is limited to transfer from alveolar regions into the pulmonary vasculature (4). Absorption studies usually employ drug dissolved in aqueous solutions, despite the fact that therapeutic aerosols often deposit drug in dry particulate form (10,11) and many hazardous environmental aerosols are highly water insoluble. Thus, xenobiotic dissolution within the respiratory tract is of importance pharmaceutically and toxicologically. Chowhan and Amaro (12) have shown that Schanker's *in situ* rat lung technique can be used to document a decreased rate of absorption when compounds are administered to the airways as free acids in suspension, as opposed to salts in aqueous solution. In the isolated lung, where absorptive transfer is easier to quantify than it is in whole animals (4), we have previously studied the behavior of solutes administered as solid aerosols when dissolution is not rate determining. The present study demonstrates how the dissolution or release kinetics of a compound may be determined from the perfusate concentration versus time profile in the isolated lung, following administration to the respiratory tract in solid particulate form. The technique has potential as a means of screening aerosol formulations for sustained-release properties within the lungs.

## THEORY

An isolated rat lung preparation developed by Byron *et al.* (4) was used to study the deposition and absorption of solid aerosols of disodium fluorescein. The dye was neither metabolized nor bound significantly to lung tissues at the doses studied. Transfer, from airways to perfusate, was controlled by pulmonary epithelial permeability and was unaffected by dissolution or perfusate flow rate. The bronchial circulation was incomplete, and dye deposition and absorption conformed to Scheme I,




Scheme I

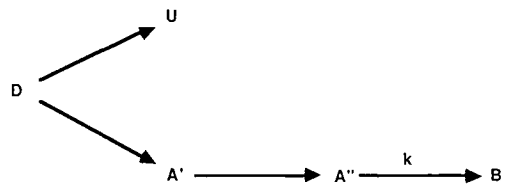
[1] University of Kentucky, College of Pharmacy, Rose Street, Lexington, Kentucky 40536.

[2] MCV, Virginia Commonwealth University, Department of Pharmacy and Pharmaceutics, P.O. Box 581, Richmond, Virginia 23231.

[3] To whom correspondence should be addressed.

0724-8741/88/0900-0574$06.00/0 

where the total deposited dose, $D$, was divided into a transferable amount, $A$, and an untransferable amount, $U$. Dissolution was assumed to be instantaneous so that $A$ could diffuse (apparently irreversibly) into the perfusate according to a first-order rate constant, $k$, which, for fluorescein, was $0.057 \pm 0.025\ min^{-1}$ and independent of dose. When a compound is formulated so that dissolution or release becomes rate determining in the absorption process, Scheme I must be modified so that the transferable dose, $A$, is split into a solid or unreleased component, $A'$, and a dissolved component, $A''$, where $A = A' + A''$ (Scheme II).




Scheme II

As undissolved drug cannot transfer, the rate of appearance of $A''$ (in the regions of the lung from which absorption can take place) will dictate the rate of its appearance in the perfusate ($dB/dt$; Scheme II). Thus, provided that a value for $k$, the rate constant for transfer, can be assigned to the absorption process, it should be possible to determine $A'$ and $A''$ as a function of time from the concentration versus time profiles obtained for $B$. The rate of appearance of $B$ is given (by definition) as

$$dB/dt = k \times A'' \quad (1)$$

If a value is assigned to $k$ and the rate of change, $dB/dt$ or $d$(concentration in perfusate $\times$ volume)$/dt$, is known (through frequent sampling and analysis of the circulating perfusate), then Eq. (1) can be rearranged to provide a solution for $A''$ (Scheme II) as

$$A'' = (dB/dt)/k \quad (2)$$

Then, provided a value is known for the total dose delivered to lung regions from which transfer is possible, $A$, the amount of undissolved material, $A'$, can be determined by mass balance from

$$A' = A - A'' \quad (3)$$

## MATERIALS AND METHODS

Disodium fluorescein (Fisher Scientific, Cincinnati, Ohio) was administered in a series of liquid bolus doses to the respiratory tract of the isolated rat lung. Four separate formulations were employed: solutions in double-distilled water and three coprecipitates of disodium fluorescein with magnesium hydroxide. The latter were prepared according to the method of Hickey and Byron (13) with the initial conditions selected to produce low, medium, and high concentrations of the dye in each of the formulations. In their publication, Hickey and Byron dried and compressed the coprecipitates in order to determine their release rates. This procedure interferes with the submicrometer size distribution (13) of the original precipitate suspension and was not employed here. Instead, the washed precipitates (13) were diluted in saturated magnesium hydroxide solution so they contained 1.4% (w/v) undissolved solids. These were stored at 4°C prior to their administration in 0.1-ml quantities to the isolated perfused rat lung (IPRL).

*Lung Preparation and Dosing.* The procedure is described in detail elsewhere (14). Briefly, adult, male Wistar rats weighing 300–350 g were anesthetized intraperitoneally with sodium pentobarbitone (60 mg/kg, 50 mg/ml; Nembutal, Abbott Laboratories, North Chicago, Ill.). The trachea was cannulated with a stainless-steel tube. On opening the thorax, the pulmonary artery was cannulated and the left side of the heart removed, allowing continuous, free perfusion of Krebs Henseleit buffer containing 4% (w/v) bovine serum albumin. The preparation was removed from the thorax and suspended horizontally in a jacketed glass chamber. The basis of dosing the lungs required expulsion of either a suspension or a solution into the airways while simultaneously inflating the lungs with a fixed volume of gas (6.1 ml). A dosing cartridge filled with 0.1 ml of the liquid dose was slipped through the tracheal cannula until its tip just projected into the rat trachea. A gas-tight seal was obtained, and a 25-μl per actuation, metered-dose inhaler (MDI) (containing fluorocarbon propellants alone) connected to the liquid-free end of the dosing cartridge. The MDI was actuated, expelling the dose into the airways and inflating the lungs reproducibly (14). One second after depressing the valve, the dosing cartridge was removed completely, allowing the lungs to deflate.

*Fluorescein Transfer from Solution.* Dye transfer to the perfusate was determined after solution bolus administration by two methods. In the first, doses ($D$; Scheme I) were delivered to the airways by syringe in 100-μl quantities with fluorescein concentrations ranging up to 1 mg/ml. This technique proved to be less consistent than desired in terms of the reproducibility with which the transferable dose, $A$ (Scheme I), could be delivered to the alveolar regions. For this reason the dosing cartridge technique (Ref. 14 and above) was employed to administer similar solution doses by simultaneous expulsion and lung inflation with fluorocarbon propellants. Samples of perfusate were removed at fixed time intervals after administration to determine their fluorescein content using a fluorimetric assay described previously (15).

*Fluorescein Transfer from Coprecipitates.* Three suspension formulations were prepared and tested. Each was administered as a 100-μl dose using the cartridge technique described above (14). Each dose contained 29.6, 55.2, and 74.1 μg (fluorescein) coprecipitated with 1.4 mg $Mg(OH)_2$. As fluorescein exists in solution together with coprecipitate, a bolus dose, $D$ (Scheme II), primed both the untransferable pool, $U$, and the transferable pools, $A'$ and $A''$, simultaneously. Sampling and assay for fluorescein were performed as for solution administration above (15). A series of five experiments was completed for each coprecipitate, and the resulting perfusate versus time profiles were compared with those collected from solution transfer experiments.

## RESULTS AND DISCUSSION

*Fluorescein Transfer from Solution.* The time dependence of dye transfer to the perfusate after 100-μl solution administrations at several different concentrations is shown in Fig. 1. The data are shown as the percentage of the trans-

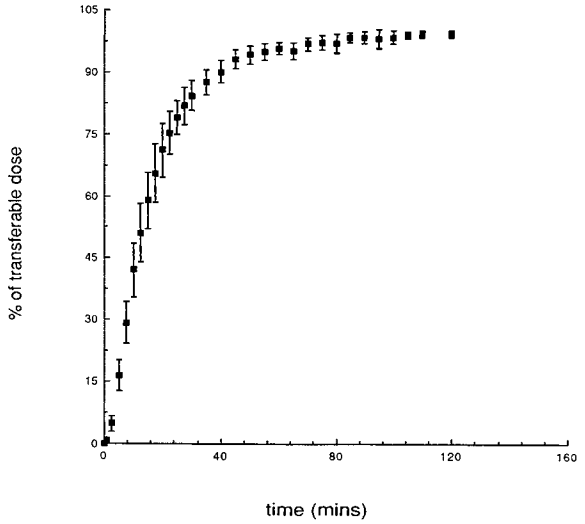



Fig. 1. Profile of the mean percentage of transferable dose versus time for fluorescein administered directly as a solution ($N$ = 12). Values for $A$ (Scheme I) ranged from ≅3 to 100 μg. The error bars are standard deviations.

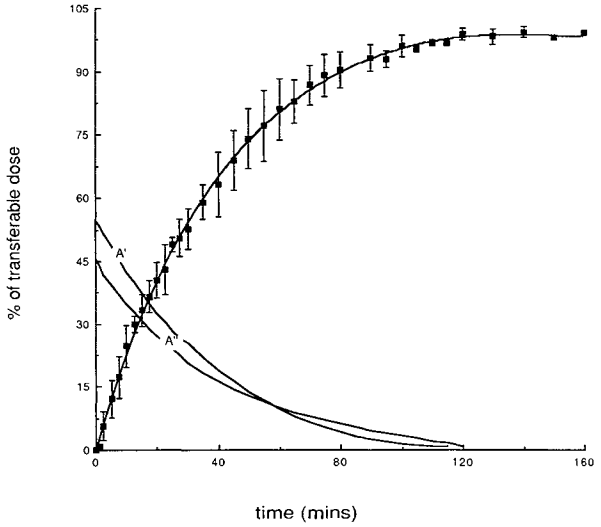



Fig. 3. The mean percentage of transferable dose (■) versus time for a magnesium hydroxide coprecipitate with fluorescein ($N$ = 3), administered as a suspension containing 29.6 μg/0.1 ml. Error bars are the experimental range. A fifth-order polynomial fit runs through the data points. Profiles for $A'$ and $A''$ were generated using Eqs. (3) and (4).

ferred dose versus time. The transferred dose was determined from the product of the concentration and volume of the perfusate in the plateau region of each transfer profile (4). At pH 7.4 fluorescein is mainly ionized and the compound is absorbed by apparent first-order kinetics. There was no difference in fluorescein absorption half-lives determined with either the dosing cartridge technique or solution instillation by syringe (Fig. 2) ($P < 0.01$). Half-lives were in agreement with those documented previously following 20 min of aerosol administration to the IPRL (12.2 ± 4.2 min) (4).

*Fluorescein Transfer from Coprecipitates.* Three precipitates were prepared to bracket the extremes of release noted by Hickey and Byron (13). The coprecipitate suspensions would always contain a quantity of free fluorescein in solution besides that associated with the $Mg(OH)_2$. Each precipitate was administered to a total of five lung preparations. When data were expressed as the percentage of the transferable dose vs time, three or four experiments from each group produced similar time profiles for dye transfer to the perfusate. These profiles are shown in Figs. 3–5. For reasons discussed later, one or two experiments from each group could not be included in these figures. Nevertheless, data from *all* experiments demonstrated slower transfer than that observed from solution (Fig. 1). When curves of $B$ vs $t$ reached a plateau, the product of the final concentration and perfusate volume (corrected for amounts withdrawn at various times throughout the experiments) provided a value for $A = A' + A''$ (Scheme II). In many cases following the

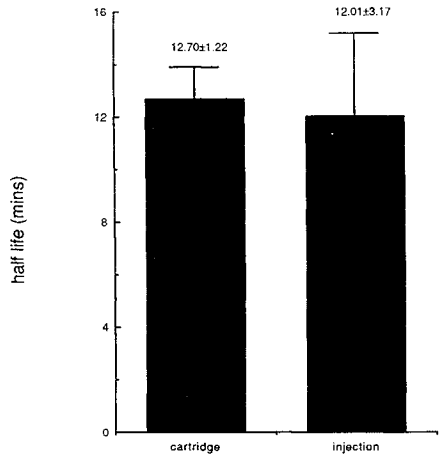



Fig. 2. A comparison of the half-lives of absorption obtained by the dosing cartridge delivery system ($N$ = 6) and simple intratracheal injection by syringe ($N$ = 6). The error bars are standard deviations. No significant difference existed between the means by Student's $t$ test ($P < 0.01$).

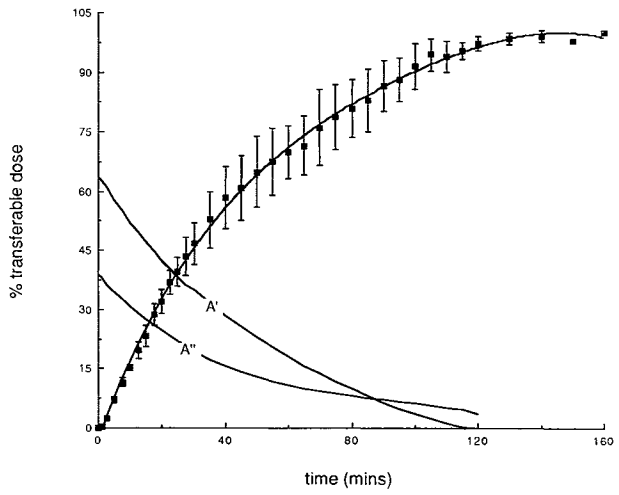



Fig. 4. The mean percentage of transferable dose (■) versus time for a magnesium hydroxide coprecipitate with fluorescein ($N$ = 3), administered as a suspension containing 55.2 μg/0.1 ml. Error bars are the experimental range. A fifth-order polynomial fit runs through the data points. Profiles for $A'$ and $A''$ were generated using Eqs. (3) and (4).

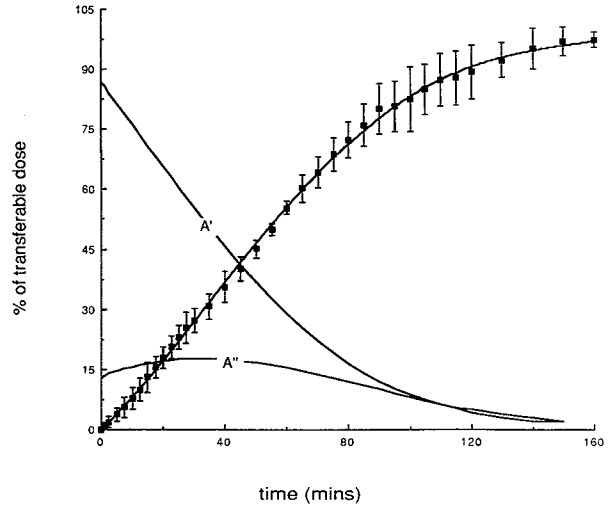



Fig. 5. The mean percentage of transferable dose (■) versus time for a magnesium hydroxide coprecipitate with fluorescein ($N$ = 3), administered as a suspension containing 74.1 μg/0.1 ml. Error bars are the experimental range. A fifth-order polynomial fit runs through the data points. Profiles for $A'$ and $A''$ were generated using Eqs. (3) and (4).

form of administration used here, the value of $U$ was negligible ($U$ was calculated from the difference in values between $B$ at the end of an experiment and $D$ at its commencement). In these cases $A$ could be determined even in the absence of a plateau. The results were consistent with a previous observation that immediately after dosing, <7% of a solution dose remained in the trachea and major bronchi when delivered in this way (14). In instances where transfer plateaued before the preparation became nonviable (4) or $U$ tended to zero (Schemes I and II), the value of the transferable dose, $A$, could be determined unequivocally. This enabled data to be presented as "percentage transferable dose" vs time [$(B \times 100)/A$; Schemes I and II]. In other cases where $U$ was significant and/or absorption appeared to continue beyond the preparation lifetime, an unequivocal value for $A$ could not be determined. As we show below, the deconvolution technique requires a known value for $A$ (Scheme I). Given that apparent first-order absorption of fluorescein occurs from solution, the rate of transfer can be described by Eq. (1). Rearranging and writing in finite terms gives

$$A'' = \left[\frac{(B_n - B_{n-1})}{(t_n - t_{n-1})}\right]/k = [\Delta B/\Delta t]/k \qquad (4)$$

where $n$ is the sample number. If the interval between sampling is short enough, then it becomes possible to estimate $A''$ and consequently apply mass balance [Eq. (3)] to obtain release as a function of time. In practice, the presence of experimental error in the raw data requires a smoothing function to prevent the generation of erratic plots of $A''$ vs $t$. To smooth the fluorescein absorption profiles, a fifth-order polynomial regression (16) was performed on each set of data, which produced close fits in all cases (smooth curves through data in Figs. 3–6). Gradients of the $B$ vs $t$ curves generated by these polynomial equations were determined at each sampling time, $t$. The rate constant, $k$, was held constant at its experimentally determined value of 0.055 ± 0.01 $min^{-1}$ (cartridge insufflation method), and $A''$ estimated using Eq. (4). Undissolved fluorescein, $A'$, was determined from Eq. (3) and all results are expressed in terms of the percentage of the total transferable dose, $A$.

The ratios of bound (solid) to free (dissolved) fluorescein in these aqueous coprecipitate suspensions vary with the dye concentrations that are used during their preparation due to complex adsorption and desorption behavior (13). In aqueous suspension these coprecipitates are therefore not an ideal dosage form. But they do constitute a means of evaluating the usefulness of the IPRL to detect dissolution determined solute transfer. The ratio of solid:dissolved fluorescein ($A'/A''$ intercept ratio in Figs. 3–6) reaching the transferable regions of the lung was, in most cases, reproducible for a chosen precipitate. These ratios increased with increasing dye content. In the case of the precipitate suspension containing 74.1 μg fluorescein (Fig. 5), solid dye reaching the transferable regions appeared to predominate, producing a biphasic time profile for $A''$. The pattern of dissolution in this case ($A'$ vs $t$; Fig. 5) appeared to mimick that predicted for particles that have log-normal size distributions (17,18), with linear release being maintained until the last particle of the smallest diameter dissolves, then the overall particle number decreasing with time.

Figures 4–6 contain data from three, as opposed to five experiments that were actually performed in each group. When data sets were rejected, in most cases this was due to the fact that an unequivocal value could not be assigned to the transferable amount, $A = A' + A''$. This occurred either

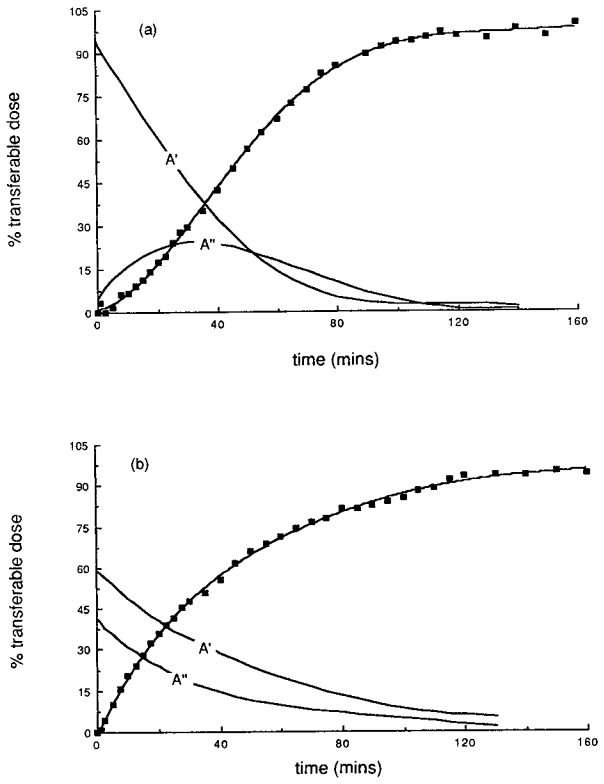



Fig. 6. Percentage of transferable dose versus time for (a) 29.6 μg and (b) 74.1 μg fluorescein in $Mg(OH)_2$ showing possible dosing aberrations.

when profiles did not plateau or when $U$ (Scheme II) was significant due to dosing difficulties or both. Other experiments that, for reasons of clarity are not reported here, consistently defied this form of data analysis because it was impossible to assign a value for $A$ due to release in the airways continuing well beyond the preparation lifetime (3 hr). Inevitable differences in dose distribution between preparations require that data are "normalized" with respect to the transferable amount, $A$, in order to avoid error accumulation in the calculated values for the amount of undissolved material remaining at a given time. If, for example, 5 μg of fluorescein was incorrectly assigned to the transferable pool, $A$, when in fact it belonged in the untransferable pool, $U$, this 5 μg would appear, incorrectly and continuously, to reside in the undissolved pool, $A'$.

On two occasions in this series of experiments, dye in perfusate vs time profiles indicated an abnormal distribution of dissolved and undissolved fluorescein among the perfused airways. There was no reason to doubt the value for the transferable amount, $A$, in either case. These data and the results of deconvolution are presented in Figs. 6a and b. In spite of attempts to ensure reproducible administration (validated for aqueous solutions) (14), it appeared that suspension dosing can sometimes lead to uneven distribution of solid and dissolved material in the perfused regions. Results of this nature should come as no surprise to workers familiar with the study and dosing of isolated organ preparations; the difficulty lies in explaining and justifying their exclusion from the main body of data. Retrospectively, there were dosing aberrations in both cases. In Fig. 6a, for example, less than 40% of the dose in the dosing cartridge was transferable, which compares with values closer to 63% for Fig. 3 and 65.9% for solutions (14). Nevertheless, it was still necessary to *presume* that the distribution of solid and solution occurred unevenly between the lung regions from which transfer was possible and those where it was not. There are several possible solutions to these problems of reproducibility. The inclusion of an internal standard alongside the test solute may enable each preparation to act as its own control. An independent assay for $A'$ and $A''$ remaining in the tissue is also desirable. In the case of isolated organ preparations such as IPRLs, the data collected during the experiment must be examined critically in order to define criteria that enable acceptance or rejection as appropriate. This population selection process is widely accepted in whole-animal studies, although no corresponding procedures appear to exist for IPRLs. While improvements continue to be made, the wide variation in experimental objectives between groups requires that each group or worker carefully define standard practices as well as quality limits for data acceptance. Although it introduces additional complexity, it is likely that the inclusion of an internal standard whose absorption kinetics are known would be a valuable improvement in experiments of this nature.

In summary, occasional dosing aberrations and the preparation's viable lifetime are the two factors constraining the use of this technique for studying dissolution or release in the respiratory tract. Problems with dosing have been largely eliminated by the use of the cartridge technique described previously (14). The present method's mathematical dependence on mass balance requires, for the collection of reliable results, that transfer is completed during the lifetime of the IPRL. This may be manifested by the existence of either a plateau in the $B$ vs $t$ profile or similar values for $B$ and $D$ (indicating that $U$ is negligible; Scheme II) at the end of an experiment. In the event that transfer is incomplete during an experiment, solute recovery from homogenized lung containing undissolved solids can result in assay problems, which we have seen in the case of some coprecipitate suspensions. While the use of whole-animal or *in situ* lung techniques may overcome some of the problems associated with the IPRL, variations in whole-body clearance, in these cases, place severe limitations on the use of absorption data to estimate dissolution or release kinetics within the airways (19). The subject is important because compounds with intermediate or low aqueous solubilities may exhibit dissolution rate-determined absorption from the lung (4,11,19–21) depending on factors such as solubility, size, surface characteristics, deposition pattern, and *in vivo* mixing dynamics.

## ACKNOWLEDGMENTS

This work was supported by an educational grant from Glaxo Inc., Research Triangle Park, North Carolina. The authors are grateful to the reviewers for their useful criticism of the penultimate paragraph in the discussion.

## NOMENCLATURE

| | |
|---|---|
| $D$ | Fluorescein dose reaching the airways |
| $U$ | Untransferable fluorescein dose depositing in upper airways |
| $A$ | Transferable dose reaching the lower airways |
| $A'$ | Solid component of $A$ |
| $A''$ | Solution component of $A$ |
| $B$ | Amount transferred or absorbed into the recirculating perfusate |
| $k$ | Apparent first-order rate constant for absorption |
| $t$ | Time in minutes |
| $\Delta B$ | The amount absorbed between sample points |
| $\Delta t$ | The time interval between sample points |
| $n$ | Sample number |

## REFERENCES

1. R. A. Brown and L. S. Schanker. *Drug Metab. Dispos.* **11**:355–360 (1983).
2. R. M. Effros and G. R. Mason. *Am. Rev. Resp. Dis.* **127**:S59–S65 (1983).
3. C. Post and A. R. Hede. *Biochem. Pharmacol.* **31**:3116–3119 (1982).
4. P. R. Byron, N. S. R. Roberts, and A. R. Clark. *J. Pharm. Sci.***75**:168–171 (1986).
5. J. A. Hemberger and L. S. Schanker. *Drug Metab. Dispos.***11**:73–82 (1983).
6. L. S. Schanker, E. W. Mitchell, and R. A. Brown. *Drug Metab. Dispos.* **14**:79–88 (1986).
7. L. S. Schanker and J. A. Burton. *Proc. Soc. Exp. Biol. Med.* **152**:377–380 (1976).
8. R. Niemeier and E. Bingham. *Life Sci.* **11**:807–820 (1972).

9. S. J. Enna and L. S. Schanker. *Am J. Physiol.* **222**:409–414 (1972).
10. P. R. Byron. *Pharm. Tech.* **11**:42–56 (1987).
11. P. R. Byron. *J. Pharm. Sci.* **75**:433–438, 1207–1208 (1986).
12. Z. T. Chowhan and A. A. Amaro. *J. Pharm. Sci.* **65**:1669–1672 (1976).
13. A. J. Hickey and P. R. Byron. *J. Pharm. Sci.* **75**:756–759 (1986).
14. P. R. Byron and R. W. Niven. *J. Pharm. Sci.* **77**:693–695.
15. A. R. Clark, P. R. Byron, and C. V. Groom. *J. Pharm. Pharmacol.* **33**:39P (1981).
16. *Statworks, Vol. 1.1,* Cricket Software, Malvern, Pa., 1985.
17. J. T. Carstensen and M. N. Musa. *J. Pharm. Sci.* **61**:223–227 (1972).
18. D. Brooke. *J. Pharm. Sci.***62**:795–798 (1973).
19. A. R. Clark and P. R. Byron. *J. Pharm. Sci.* **74**:939–942 (1985).
20. A. J. Hickey and P. R. Byron. *Drug Des. Deliv.* **2**:35–39 (1987).
21. P. R. Byron and A. R. Clark. *J. Pharm. Sci.* **74**:934–938 (1985).