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Drug Delivery via the Respiratory Tract

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ABSTRACT

Inhalation offers an enormous absorptive surface area for rapid drug absorption and substantial absorption of polypeptides. Due to slow clearance from the lower lung, even compounds with very small absorption rates can be absorbed in significant quantities over 10-12h periods. Aerosol dosimetry problems can also be minimized when lung-normal patients are considered. In the near future, optimal formulations will be combined with modified aerosol delivery devices to achieve reproducible dosing. These will be used as alternatives to parenteral delivery for drug doses of the order of milligrams or less. Research on the molecular structural dependence of lung disposition is in its infancy. Absorption kinetics for small molecules are known to depend on lipophilicity and molecular size. For macromolecules however, electronic charge and site of deposition may be additional determinants of bioavailability. Carrier-mediated absorption processes may also be important. The pulmonary absorption of a number of molecules is reviewed with special emphasis on new and promising products of biotechnology like human insulin and human growth hormone. Delivery improvements in the future should ensure, ideally, that nondenatured, monomeric pure compounds are delivered reproducibly and predominantly to the lung itself, so that these compounds may elicit reproducible systemic effects following absorption.

INTRODUCTION

Drug delivery via the lung is receiving considerable attention for several reasons (Ahmed, 1990). Difficulties associated with the systemic delivery of peptides, polypeptides and other macromolecules, coupled with the likelihood of reasonable values for their pulmonary bioavailability has led to interest in their presentation for absorption via the lung. Also, the lung's huge surface area for absorption (approx 75 m^2 [Hollinger, 1985]) means that systemic side effects can be significant even when compounds are administered in small doses for local effects (Moren et al., 1985). The former subject of polypeptide bioavailability following aerosol administration is a significant new field of research upon which we will center this article. Fortunately, many of the aerosol deposition

pulmonary drug delivery; aerosol inhalation; lung absorption; peptides; proteins; macromolecules

abnormalities associated with delivery of small molecules intended for local effects within the lung can be neglected; products to be administered for systemic effect will almost certainly be administered to a lung-normal population. Total deposition in the lungs of normal humans for well characterized particulate aerosols shows a coefficient of variation of the order of about 10% (Chan and Lippmann, 1980; Gerrity, 1990), a value which is in keeping with our concepts for reproducible dosing within the pharmaceutical sciences. Nevertheless, if inhalation is to be used for systemic delivery purposes, the drug formulator and product designer must try to deliver a reproducible dose to the lungs of the patient. Thus, the issues associated with improving the reproducibility of deposition in this journal (Clark, 1993) are all relevant here. Many important points relating to dosimetry have also been discussed in previous articles by us (Byron, 1990a, 1990b, 1990c; Patton and Platz, 1992) and will not be repeated here. This article will begin by presuming the possibility of designing and producing better formulations and aerosol generators, which minimize variations in the deposited dose.

EXTRAVASCULAR DRUG DELIVERY

Pharmacokinetics

While the complexities of distribution, metabolism and excretion (DME) are drug-dependent and, to large extent, beyond pharmaceutical control, the simple plasma drug concentration versus time profile resulting from first-order absorption and elimination is shown in Figure 1. Parameters which are subject to pharmaceutical control, which influence the shape of this and subsequent profiles, include D, F, k_a, t_{lag} and τ, where t_{lag} is discussed below, τ is the interval between doses and D, F and k_a are as defined in Figure 1. All these parameters are concerned with drug input to the circulation and can be manipulated. DME, or the intrinsic pharmacokinetics of the compound on the other hand, is out of pharmaceutical control in the sense that the body will deal with drug, once absorbed, in its own way and in ways dependent upon the chemical nature of the drug itself. For a given drug, increasing the value of FD will increase the area under the concentration-time profile (AUC) proportionally. Increasing values of k_a (FD held constant) result in decreasing values for t_{max}, the time at which plasma concentration passes through a maximum, and increasing values for C_{max}, the concentration at that maximum, in such a way that AUC remains constant. In chronic repetitive dosing regimens, the value of the drug's

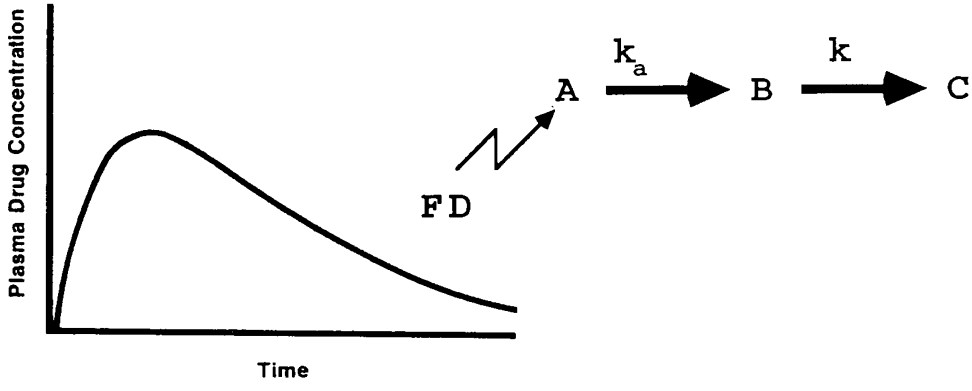


FIGURE 1: A typical plasma concentration versus time profile resulting from first-order absorption and elimination of a compound. The bioavailable dose (bioavailable fraction, F x administered dose, D, = $FD=A_0$) is absorbed from the depot, A, into the body, B, according to a first-order rate constant for absorption, k_a, commencing at time zero; k is the first-order rate constant for drug elimination from the body. Amounts of drug in A, B and C are time dependent. Plasma concentration is considered to be directly proportional to the amount in B.

input rate to the systemic circulation, FD/τ, defines the range of drug concentrations in plasma, between which oscillations occur, once "steady state" has been achieved. The subject of pharmacokinetics is treated in depth by a number of excellent texts (e.g., Rowland and Tozer, 1989; Notari, 1987). This brief introduction to the subject has been included here to illustrate the importance of controlling the input factors k_a, F, D and τ, if we are to hope to control the resultant plasma concentration versus time profiles.

One final drug input term, t_{lag}, is a parameter which describes the lag time between drug administration and absorption commencement. This term is often important for orally administered compounds where esophageal transit and even gastric emptying must often occur before the drug is at the absorption site. Because solid dosage forms must then release the drug prior to absorption, t_{lag} can be substantial in some cases. It is probably not a significant issue in the case of drug administration by aerosol. Although aerosol generation, inhalation and deposition are actually sequential, they occur over such a short time frame (seconds) that they can be considered to be simultaneous events. Furthermore, even in cases where lipophilic compounds are administered in powder form, respirable particles have a very high surface/volume ratio and, as a result, tend to dissolve rapidly (Byron, 1990c). Unless some novel formulation approach has been adopted capable of increasing the value of t_{lag} therefore (Lai et al., 1993), we may neglect this term in our discussion. For similar reasons, the value of the dosage interval, τ, will not be discussed in the remaining text. This term can be adjusted during the clinical evaluation of a compound, once the dosage form has been fixed. The primary determinants of bioavailability, the fraction absorbed, F, and the absorption rate constant, k_a, will be the focus of this presentation. Specifically, we will discuss factors controlling absorption from the lung and attempt to assign a limit to the size of molecules which can be absorbed in a realistic time frame. To control and perhaps enhance values for F and k_a in accord with the philosophy introduced above, should enable researchers and product developers to control the shape of plasma concentration versus time profiles; hence, to ensure that values for C_{max}, t_{max} and AUC are sufficiently reproducible to enable reliable systemic therapy to result following aerosol administration.

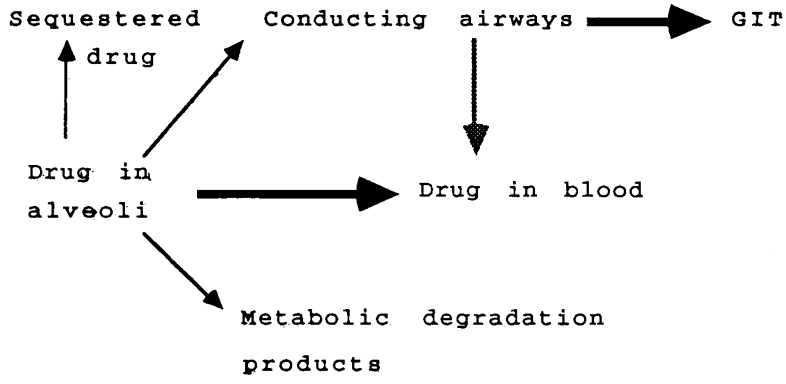
PULMONARY ABSORPTION

Delivery of drug within a fixed dosing range to the lumen of the gastrointestinal tract (GIT) is not usually a problem. Thus, the factors controlling the bioavailable dose of an orally administered drug are often described as those which represent alternative pathways for drug disappearance. In the lung-normal individual, there is reason to believe that there are fewer complications likely to interfere with the absorption process than there are in the GIT. In this respect, inhalation may offer fairly reproducible absorption kinetics and thus have some advantages over oral administration.

Obvious differences between the lung and the GIT include the lack of dietary complications in the lung, the low levels of extracellular enzymes (Gonda and Byron, 1978; Crooks and Damani, 1990) and the absence of quantitative and qualitative interindividual metabolic differences due to the presence of different flora and fauna in the respiratory tract.

Absorption Determinants

The key issues affecting pulmonary bioavailability are shown in Scheme 1. Both the conducting airways and the alveoli are likely to show significant rates of drug absorption. The greater importance of the alveolar regions stems mainly from the fact that mucociliary clearance is considerably slower from the periphery than it is from the upper airways (Byron, 1986). For larger molecules, the increased duration of residence in the lower lung should lead to a greater opportunity for absorption from the alveoli. Gas exchange also occurs primarily in the lower lung but this is because of the existence of concentration gradients in these regions (the pulmonary blood supply differs from the bronchial (Hollinger, 1985) in that the former is largely deoxygenated while the latter is oxygenated, deriving from the aortic arch). In Scheme 1,



SCHEME 1

mucociliary clearance to the GIT is considered to represent drug loss, as is metabolic clearance and sequestration in various cell types and membranes (Crooks and Damani, 1990; Byron and Phillips, 1990). It is clear from Scheme 1 that the relative rates of the various processes will define the bioavailable fraction of the dose. While there is discussion of prolonged acting microparticulates by drug release control (Byron and Phillips, 1990; Lai et al., 1993), this will be neglected here. It is clearly premature to attempt to control the release rate of a compound, when its bioavailability may already be poor due to slow absorption and high rates of parallel clearance.

Understanding those factors which control pulmonary absorption kinetics is obviously the key to enhancing bioavailability. In a recent book (Byron, 1990d) the molecular dependence of lung binding and metabolism was considered alongside the parallel processes of absorption, clearance and dissolution in the lung (Byron and Phillips, 1990). Key features of this work will be repeated and amplified as it relates to the systemic delivery of new products of biotechnology via the lung.

Various experimental models have been used to study pulmonary absorption kinetics making it difficult to compare results between research groups. For this reason, and the fact that our own work employs the rat, the data presented in Table 1 is restricted to that collected using the in situ rat lung technique by Schanker's group (Lanman et al., 1973; Enna and Schanker, 1972; Burton and Schanker, 1974a and 1974b; Burton et al., 1974; Schanker and Burton, 1976; Schanker and Hemberger, 1983; Brown and Schanker, 1983).

The upper part of the absorption model and the first term on the right hand side of the equation shown in Figure 2 represents absorption proceeding according to passive diffusion via partitioning through cell membranes. If this process is assumed to dominate (the terms for endocytic and junctional transport tend to zero), it enables the derivation of an equation for the apparent first-order rate constant for absorption, k_a, of a given solute in the airways as:

$$k_a = D_M A_M K_D / (h V) \qquad (1)$$

The parameters D_M, A_M, K_D, h and V are, respectively, the solute's diffusion coefficient through the pulmonary membranes (the epithelium is considered to be rate determining (Effros and Mason, 1963), the total membrane surface area available for absorption, the solute's membrane/donor phase partition coefficient, the thickness of the membranes (the diffusion barrier is believed to be $<<1$ μm in the alveoli [Hollinger, 1985]) and the fluid volume in the airways in which the dose, D, is dissolved. Table 1 reports the half-lives for absorption ($0.693/k_a$) for a range of small molecular weight, MW, compounds with different apparent partition coefficients, K_D, (chloroform/water at pH 7.4). The fourth and fifth columns of Table 1 (K_D/MW^n), when compared to the ranking of absorption half-lives in column 2, show the predictability of equation 1. Either the Stokes-Einstein equation or the Wilke-Chang relationship can be used to predict changes in diffusion coefficients with solute molecular weight, MW (Byron et al., 1981). Incorporating this prediction in equation

TABLE 1

Experimental Half-Lives, $t_{0.5}$ (mins), for Pulmonary Absorption in the Rat Lung Ranked versus the Ratio of Solute K_D/ (MOLECULAR WEIGHT)^n. Absorption data are all from articles by Schanker's group. The value of n (Equation 2) is shown using diffusion coefficients predicted by Stoke's-Einstein equation (n = 0.33) and the Wilke-Chang relationship (n = 0.56).

Solute	$t_{0.5}$	MW	$K_D/MW^{0.33}$	$K_D/MW^{0.56}$	K_D
Antipyrine	<1	188	41,720	12,731	239,000
Pentobarbital	<1	226	37,759	11,052	230,000
Phenobarbital	<1	232	4,101	1,193	25,200
Sulfadimethoxime	<1	310	3,317	904	22,450
Sulfamethoxypyridazine	<1	280	2,247	626	14,700
Isoniazid	1.9	137	563	184	2,900
Chloramphenicol	1.9	323	358	97	2,460
Procainamide	3.2	235	324	94	2,000
Sulfisoxazole	3.3	267	26	7.4	168
Doxycycline	7.8	444	494	124	3,769
Erythromycin	11.9	734	1,394	392	12,578
Tetracycline	13.9	444	164	41.4	1,257
Benzylpenicillin	31.2	334	13.8	3.7	96
Sulfaguanidine	41.6	232	4.6	1.3	28
Ethambutol	41.6	218	2.0	0.6	12.4
Ouabain	69.3	585	4.5	1.1	37.6
Dihydroouabain	83.2	587	1.0	0.2	8.1
Sulfanilic acid	46.2	174	0.04	0.01	0.2
Tetraethylammonium	69.3	130	0.3	0.25	3.9

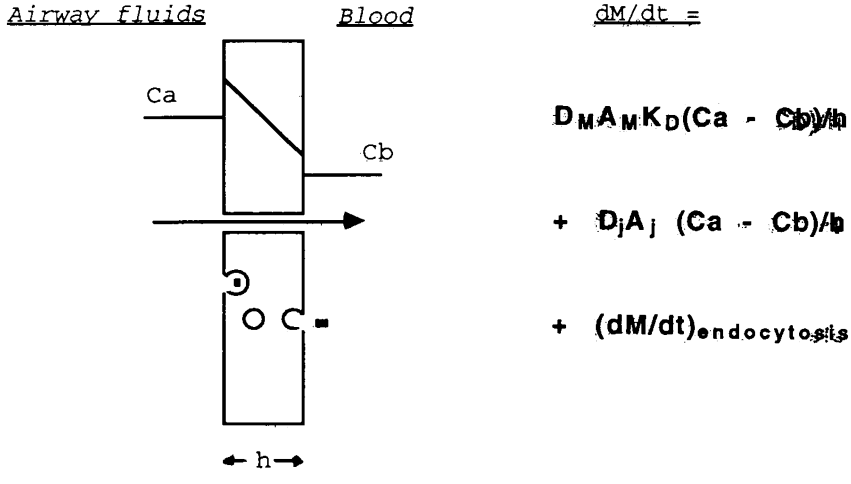


FIGURE 2. A model for transpulmonary solute diffusion from airways to blood through a membrane of thickness, h. Concentrations in the airway and blood are Ca and Cb. Also shown diagrammatically are transport through tight junctions and transport via endocytosis. All pathways are theoretically available to all solutes. The rate of mass transfer, dM/dt, is shown for each of the processes in turn, the sum providing the total flux through the membrane. The subscript j refers to tight junctional transport; other terms are defined in the text.

1 and assuming that the membrane thickness, h, fluid volume, V, and surface area, A_M, remain constant for the fixed experimental technique used by Schanker's group gives

$$t_{0.5} = A / (K_D / MW^n) \qquad (2)$$

where A is a proportionality constant. The value of the power, n, varies depending on the use of the Stokes-Einstein (n =0.33) or the Wilke-Chang (n=0.56) relationship to predict D as a function of MW (Byron and Phillips, 1990). Table 1 also shows the rapidity of absorption for small molecules when the kinetics are likely to become perfusion rate limited. When small solutes are sufficiently lipophilic, transfer rates are of the order of seconds and absorption rates are defined by blood flow rates through the pulmonary vasculature (Effros and Mason, 1983). The model-derived equation 2 fails to account either for endocytosis or for diffusion through aqueous channels between tight cellular junctions in the pulmonary epithelia. These processes are both described by Effros and Mason (1983), and others, and are illustrated diagrammatically in the lower portions of Figure 2. Even the more complete description afforded by Figure 2 and its accompanying equation is simplistic however. Active or carrier-mediated processes are neglected as are boundary layers, and the poor predictability of diffusion coefficients in membranes creates further problems. Nevertheless, the apparent inverse relationship between the theoretical (K_D / MW^n) and the experimental value for $t_{0.5}$ gives some general support to the partitioning absorption model shown in Figure 2 and applied in Table 1.

Table 2, on the other hand, shows the molecular weight dependence of absorption, again from Schanker's data but extended to consider the absorption of a limited number of macromolecular solutes. In this case the table is restricted to those hydrophilic solutes which may pass either via tight junctions in the epithelial cell layer or by endocytosis (Byron and Phillips, 1990). Other work on the pulmonary absorption of macromolecules is sparse. While there is information in the literature on the apparent permeability of pulmonary membranes to molecules like transferrin and ferritin (Huchon et al., 1987; Williams, 1984a and 1984b; Williams, 1987), the data is difficult to compare to that shown in Tables 1 and 2. Accordingly, we have begun to perform work using the rat lung as a macromolecular drug absorption model. Some of our experiments have been designed to assess the dependence of pulmonary absorption kinetics on macromolecular structure (Niven et al., 1990) and formulation (Niven and Byron, 1990). Hydrophilic synthetic polypeptides (poly - [hydroxyethyl] - aspartamides, PHEA) have been chosen which are resistant to metabolic breakdown and carry a fluorescent label for ease of assay. The net absorption rate of different polydisperse PHEA molecular weight distributions (MWD) was studied and found to decrease with increasing molecular weight. Absorption was characterized with time after dosing to the airways of the isolated perfused rat lung as aqueous sprays (Byron and Niven, 1988). Interestingly, PHEA's absorption

TABLE 2

Absorption Half-Lives of Some Lipid Insoluble (low K_D) Compounds Alongside Some Hydrophilic Macromolecules. Absorption data are all from articles published by Schanker's group using the in situ rat lung model.

Solute	$t_{0.5}$ (min)	MW (D)
Procainamide ethobromide (PAEB)	70.0	264
N-Acetyl PAEB	38.5	306
Sucrose	87.0	342
Tc-Diethylenetriaminepentaacetate	30.9	492
Cyanocobalamin	180.0	1,355
Inulin	225.0	5,250
Dextran	688.0	20,000
	1670.0	75,000

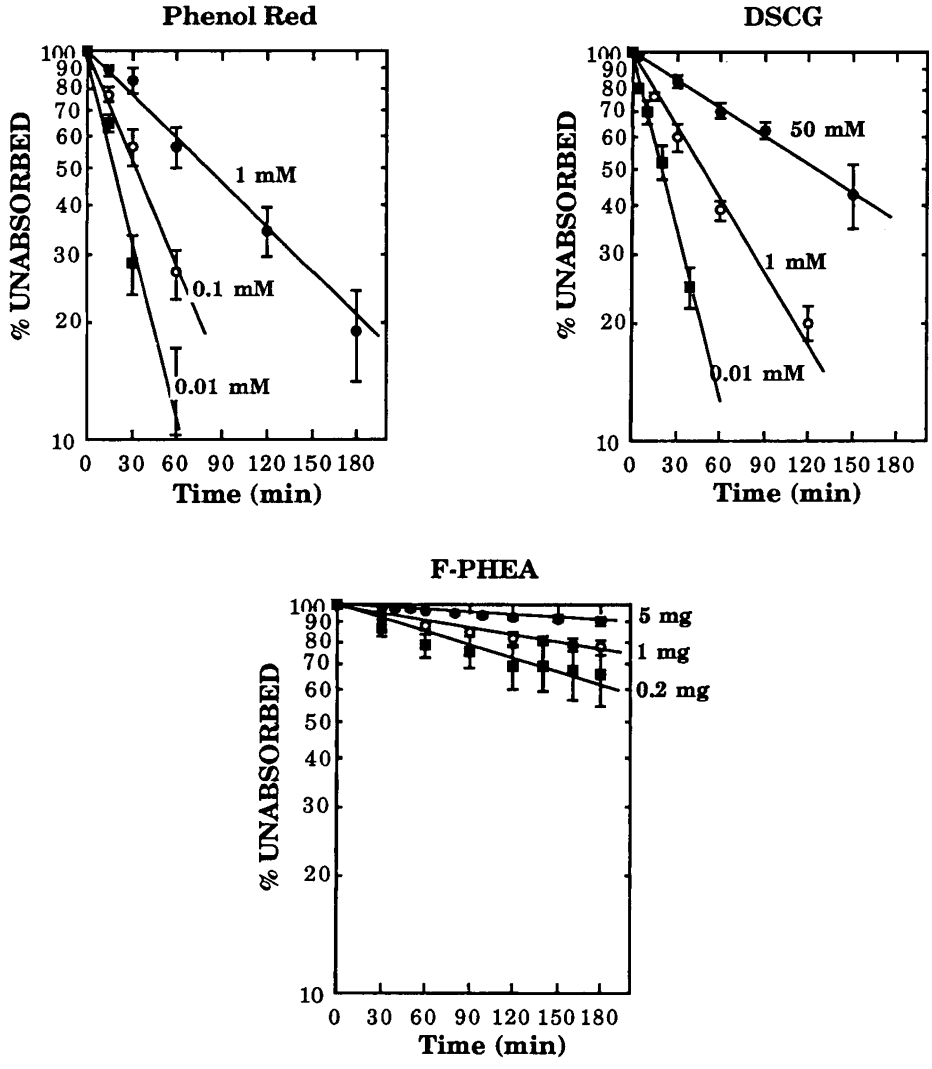


FIGURE 3. Percent unabsorbed versus time for phenol red (data from Enna and Schanker, 1973), disodium cromoglycate (DSCG; data from Gardiner and Schanker, 1974) and fluorophore labelled (M_W = 8.6 kD) PHEA (Byron et al., 1993) in the rat lung. The three curves in each case show the data following administration of different doses as different solution concentrations (Phenol red and DSCG) and different nominal doses (PHEA). Carrier-mediated absorption was apparent in all cases.

kinetics showed a dose dependency rather like that reported for disodium cromoglycate or phenol red in the in situ rat lung (Enna and Schanker, 1973; Gardiner and Schanker, 1973). The results are shown in comparative form in Figure 3. These degrees of dose dependence (Figure 3) when taken alongside the fact that doses administered by inhalation are often small, may mean that enhanced bioavailability of small metered doses could well result from the presence of, as yet, ill-defined carrier systems in the lung.

While all the MWDs (up to 40 kD) of PHEA which we have delivered to the isolated rat lung have been absorbed to some degree, there is a sieving effect which differentiates between small and large molecules in terms of the rates at which they are absorbed. More recently, we have been able to show that at early times after PHEA administration to the airways, the absorbed material is richer in smaller molecular weight polymer. In a recent study, PHEA with M_W = 8600 and M_n = 5300

Daltons (weight and number averaged molecular weights, respectively) was about 7% absorbed after 100 min in 8 rats at one dose level (approximately 3 mg). The MWD of the absorbed PHEA at this time was characterized as M_W = 6670 ± 526 and M_n = 4680 ± 640 Daltons. In fact, there appears to be some change in absorption mechanism at or around a molecular size of about 7 kD for PHEA, where absorption kinetics show a discontinuity (Byron et al., 1993). This does not mean that molecules with sizes greater than 7 kD cannot be absorbed at significant rates. Even PHEAs with much larger MWDs were absorbed continuously by the rat lung (Niven et al., 1990; Byron, 1990).

Very recently (Patton and Platz, 1992; Patton et al., 1993), the bioavailability of interferon alpha, calcitonin and parathyroid hormones of widely different molecular weights were determined in the rat following intratracheal instillation. This work showed quite clearly, that significant pulmonary absorption occurs in the rat for interferon alpha (F > 0.56) and others. This molecule ranges from 19-22 kD in size depending on whether or not it is glycosylated. Importantly, however, the value of t_{max} (Rowland and Tozer, 1989)

$$t_{max} = \frac{2.303 \log_{10} (k_a/k)}{k_a - k} \qquad (3)$$

the time at which plasma concentration reached a maximum, was significantly increased for peptides with larger molecular weights. Equation 3 is correct if the absorption and elimination scheme in Figure 1 is valid and k is the compound's first-order elimination rate constant. The equation shows the relationship between t_{max} and the k_a/k or input/output ratio. Provided therefore, we make the assumption that values for the elimination rates, k are of similar orders of magnitude, t_{max} should provide some indication of the relative values for k_a between compounds. This relationship, while theoretically fragile, is illustrated in Table 3 for a range of polypeptides with increasing molecular weights (Patton et al., 1993). The general trend in this data demonstrates the likely prolongation of plasma concentration versus time profiles which we should expect as molecular weight is increased. This has nothing really to do with formulation but is rather a property of the molecule. While small molecules delivered by aerosol may well provide plasma concentration versus time profiles which can almost duplicate intravenous curves (Byron and Clark, 1985; Clark and Byron, 1985), as molecular size is increased absorption rates decrease substantially. It is currently not well

TABLE 3

Molecular Weights (MW) and Values for t_{max} for a Range of Polypeptides

Polypeptide	MW (Daltons)	t_{max} (hr)
LHRH	1,067	1
DDAVP	1,209	0.5
Calcitonin	3,418	0.25
PTH[a]	4,109	0.25
Insulin	5,700	0.25
GCSF	18,600	1.5
Interferon alpha[b]	19,000	6
hGH	22,000	0.75
Alpha 1-antitrypsin[b]	45,000	12
Alpha 1-antitrypsin[c]	51,000	48
Albumin	68,000	20
lgG	150,000	16

[a] parathyroid hormone, 34 amino acid active fragment.
[b] natural, glycosylated.
[c] E.coli derived, nonglycosylated.

understood, if larger proteins (>20-30kD), whose absorption rate constants dictate that they be absorbed from the lung over a period of many hours or several days, can have high systemic bioavailabilities. This, because the clearance mechanisms for soluble proteins, other than absorption, from the alveoli are poorly researched.

With the data from Table 3 and the argument above in mind, Table 4 is a more extensive and speculative list of biotechnologic products which could feasibly be delivered by inhalation. The table shows compounds in 4 major categories alongside an abbreviated list of indications. These compounds are only 28 of the hundreds which are currently under investigation by the industry. Data in the column titled "animal bioavailability" indicates that pulmonary studies have been performed. Bioavailability is then expressed in terms of the percentage of the dose (actually deposited in the lung) which was then absorbed. Many of these compounds have poor nasal absorption (in the absence of absorption promoters) and could reasonably be administered to the lung without adjuvants. Human feasibility trials have already been performed in some cases. The column showing the likely duration of therapy provides some indication of the magnitude of toxicity testing required prior to marketing a dosage form for inhalation. In previous articles we have already reviewed the immunologic issues associated with chronic macromolecular delivery to the lung (Byron, 1990c; Patton and Platz, 1992). The outcome of these reviews indicated that aerosol therapy required the deposition of undenatured human recombinant macromolecules whenever these were to be administered chronically. Fortunately, this appears to be possible with DNase and other proteins even when these are administered via nebulizer (Cipolla et al., 1993; Dalby et al., 1992). Provided aggregated, denatured insoluble and impure materials can be avoided, immunologic responses do not appear to be a problem when endogenous materials are to be administered by inhalation (Byron, 1990). This does not mean however, that all the compounds listed in Table 4 will be found suitable for aerosol administration. Other obstacles may be a compound's promotion of an undesirable pharmacologic effect, overly rapid pulmonary metabolism and a requirement for highly specific regional deposition in the lung.

Other Obstacles

Many "broad spectrum" peptidases exist in lung tissue (Altieri, 1991) e.g., "enkephalinase" or neutral endopeptidase, cell secreted enzymes like chymase and tryptase (from mast cells) and elastase (from neutrophils). As a result, not all small peptides have good lung bioavailability. The pentapeptide leu-enkephalin is rapidly metabolized during its absorption from the airways in the isolated rat lung (Gillespie et al., 1985; Llorens and Schwartz, 1981). Vasoactive intestinal peptide (VIP; 28 amino acid residues) has a bioavailable fraction F, < 0.01 (Thomson, 1991; Altieri, 1991). The study of peptidergic systems in the airways is a new branch of bronchopulmonary pharmacology and VIP is a good example of a peptide which has proven pharmacologic effects in the lung (Altiere, 1991). It potentiates bronchodilator activity and this effect is more pronounced during influenza infections in animals and when VIP is administered in the presence of peptidase inhibitors. These results imply that metabolism kinetics themselves can dictate the magnitude and duration of VIP's local effects within the lung. In our work in the isolated lung (Byron et al., 1991) polyhydroxyethylaspartamide was shown to be minimally metabolized during absorption. This was believed to be due to its unnatural stereochemistry in the aspartate moieties (Niven et al., 1990; Byron et al., 1991). This rather resistant structure is unlikely to be typical of biologically active polypeptides. However, pulmonary metabolism studies are usually performed either in cell homogenates or following delivery direct to the pulmonary blood supply and endothelia. Enzyme systems and their locations in the lung have not been studied widely, nor are there many appropriate models other than isolated or in situ lung systems for screening metabolism during absorption. In short, it is difficult to say whether pulmonary metabolic clearance (Scheme 1) will or will not be a problem unless a molecule is screened for its bioavailability following aerosol or intratracheal delivery. While metabolism at the site of absorption does not preclude peptide delivery by aerosol, extreme variability in the rate of such metabolism would be cause for concern. The propogation of pharmacologic effects within the lung itself may also be a reason for discontinuing inhalation studies of a compound intended for systemic administration via the lung.

TABLE 4a

Biotechnologic cytokines and hormone products which could feasibly be delivered via the lung

MACROMOLECULE	APPROX. MW	INDICATIONS	THERAPY DURATION	ANIMAL BIOAVAIL.	HUMAN FEASIB.	REFS[a]
CYTOKINES						
Interferon-alpha	19-22 kD	Hepatitis, cancer	MONTHS	>56%	YES	1-5
Interferon-beta	19-22 kD	Multiple sclerosis	YEARS			6
Interferon-gamma	18 kD	Chronic granulomatous dis.	YEARS			7,8
Interleukin 2	18 kD	Renal cancer	MONTHS		YES	9
Interleukin 6	18 kD	Thrombocytopenia	WKS-MTHS			
Interleukin 11		Thrombocytopenia	WKS-MTHS			
Interleukin 12		T-cell disorders	WKS-MTHS			
Interleukin 1 RA[b]	18 kD	Rheumatoid arthritis	WKS-MTHS			
Macrophage CSF[c]	80 kD	Infections due	WKS-MTHS			
Granulocyte CSF	18 kD	to cancer	WKS-MTHS	62%		10.11
GM CSF	18 kD	chemotherapy	WKS-MTHS			12
Erythropoietin	30 kD	Anemia	MTHS-YRS			
HORMONES						
Calcitonin	3 kD	Pagets dis., osteoporosis	YRS	17-67%		5
Parathyroid hormone	4-9 kD	Osteoporosis	YRS	40%		5
Insulin	6 kD	Type I/II diabetes	YRS	56%	YES	13-21
Amylin	4 kD	Type I diabetes	YRS			
Growth hormone	22 kD	Short stature, wasting	YRS	6-40%		30-32

[a] Table 4c. [b] RA = Receptor antagonist [c] CSF = colony stimulating factor

TABLE 4b

Biotechnologic peptide derivatives and carbohydrate products which could feasibly be delivered via the lung

MACROMOLECULE	APPROX. MW	INDICATIONS	THERAPY DURATION	ANIMAL BIOAVAIL.	HUMAN FEASIB.	REFS[a]
PEPTIDE DERIVATIVES						
LHRH agonist	1 kD	Infertility, endometriosis	MONTHS	>95%	YES	33-35
Amylin antagonists	1-2 kD	Type II diabetes	YRS			
Somatostatin agonist	1 kD	Cancer, diarrhea of AIDS	MTHS-YRS			
IIBIIIA antagonist	1 kD	Angina - thromboembolisms	WKS-MTHS			
ACTH 4-9[b]	1 kD	Cisplatin-induced neuropathies	WKS-MTHS			
GRF agonist 11	1 kD	Short stature, wasting	MTHS-YRS	40%		36
Bombesin antagonist	1 kD	Lung Cancer	WKS-MTHS			
Hirulog or hirudin	1 or 7 kD	Antithrombotics	WKS-MTHS			
DDAVP	1 kD	Antidiuretic	YRS	20-45%		37
CARBOHYDRATES						
Heparin	15-20 kD	Antithrombotic	WKS-YRS		YES	38-44
Low MW Heparin	3-5 kD	Antithrombotic	WKS-MTHS			

[a] Table 4c
[b] amino acids 4 through 9

TABLE 4c

Authors and Study Dates From Tables 4a and 4b.

REF.#	AUTHORS	DATE	REF#	AUTHORS	DATE
1.	Maasilta et al.	1991	23.	Guerra and Kitabchi	1976
2.	Kinnula et al.	1989	24.	Berger et al.	1982
3.	Van Zandwijk et al.	1990	25.	Murat and Slama	1985
4.	Kinnula et al.	1990	26.	Edsberg et al.	1987
5.	Patton et al.	1993	27.	Roy et al.	1980
6.	Platz et al.	1991	28.	Ellenberg and Rifkin	1983
7.	Eisenberg et al.	1991	29.	Dimitriadis and Gerish	1983
8.	Debs et al.	1988	30.	Patton and Platz	1992
9.	Huland and Huland	1990	31.	Patton et al.	1990
10.	Niven et al.	1993	32.	Folkesson et al.	1992
11.	Platz et al.	1992	33.	Adjei and Carrigan	1992
12.	Rose et al.	1992	34.	Adjei and Garren	1990
13.	Colthorpe et al.	1992	35.	Adjei et al.	1990
14.	Sakr	1992	36.	Smith et al.	1993
15.	Liu et al.	1993	37.	Folkesson et al.	1990
16.	Yoshida et al.	1979	38.	Rosner	1965
17.	Gansslen	1925	39.	Hellgren et al.	1981
18.	Wigley et al.	1971	40.	Mahadoo et al.	1980
19.	Elliott et al.	1987	41.	Mahadoo et al.	1981a
20.	Kohler et al.	1987	42.	Mahadoo et al.	1981b
21.	Laube et al.	1993	43.	Mahadoo et al.	1981c
22.	Ziel et al.	1987	44.	Bick and Ross	1985

Aerosol delivery problems themselves are naturally not trivial issues. This is especially true when blood levels must be controlled fairly carefully. The literature surrounding insulin and leuprolide is a valuable and instructive resource in this respect.

Aerosol Insulin

A variety of human and animal studies conducted by others have shown that simple insulin formulations are well absorbed by the lungs. Surprisingly, there is more human than animal data. Absolute bioavailabilities of pulmonary insulin in animals are >50% based on amount delivered to the lungs (Yoshida et al., 1979; Colthorpe et al., 1992; Sakr, 1992; Liu et al., 1993). However, in the human studies, comparisons were made based on the amount of material placed in the aerosol

device and were thus lower and in the range of 20-25% because of losses in the device and mouth of the patients. There have been five independent human studies that show that treatment of diabetes with aerosol insulin is feasible (Table 5). They were all brief studies of several days duration.

The first demonstration of the efficacy of aerosol insulin was made in Germany in 5 adult diabetic patients (Ganssler, 1925). At approximately 2.5 hrs after inhaling 30-50 "Einheiten Insulin," blood glucose levels had fallen by 26% (25.9 ± 7.6% standard deviation). Presumably, a relatively impure animal insulin was used. In 1971, Wigley et al. verified that aerosol insulin lowered blood glucose in rabbits, then they tried it on themselves (3 normal subjects). They used regular pork-beef insulin (U-500, Eli Lilly) in a Devilbiss No. 40 glass nebulizer which produced a mean particle size of ~ 2 μm. The normal subjects inhaled the insulin for 2-10 minutes and their blood glucose levels all fell within 30 minutes from about 85 mg/100 ml to about 65 mg/100 ml. They then did a more detailed study with four adult diabetic patients and measured plasma immunoreactive insulin (IRI). The absorption was rapid but variable with an increase in plasma IRI detected as early as 15 minutes in one patient and peaks occurring within 30-60 minutes. In one patient, the decrease in plasma glucose was as rapid as that seen following IV administration. The variability in the shape of each subject's plasma IRI profile was in part caused by variable cooperation of the patients and lack of training with the nebulizer. Elliott et al. (1987) using Actrapid, semi-synthetic human insulin (U-500, Eli Lilly) and a patient activated nebulizer to deliver uniform doses (±5%), performed a 3-day study in six diabetic children. One child continued to use aerosol administration at home for six months (Elliott, personal communication). Blood glucose control was at least as good as a control day when they received their usual dose of subcutaneous insulin. The aerosol dose was given before each meal in 30-50 breaths (~2μl/breath) from the nebulizer. The efficiency of absorption in non-diabetic subjects was between 22-25%, but was difficult to estimate in diabetics because of anti-insulin antibodies and poor matching of results on the day subcutaneous insulin was given as compared to the day nebulized insulin was delivered. The authors stressed that efficiency and reproducibility of delivery could be improved by optimizing compressor flow rates and the level to which the nebulizer bowl was filled (to obtain optimal particle size nebulae); also by regularizing the depth and duration of inspiration. Also in 1987, Kohler et al. used aerosol insulin to measure lung permeability in 5 healthy smokers and 7 healthy non-smokers in Germany. Like Elliott, they developed a new nebulizer device. They state that their device produced "a monodispersional (particle size <2.5μm) aerosol." Subjects inhaled 23.1±2.8 U human insulin (U-500 Eli Lilly). The peak of insulin in the peripheral blood was achieved in both groups 15 minutes after inhalation: 45.6±9.8 μU/ml in the 5 smokers, 13.6±1.1 μU/ml in the 7 non-smokers (±SEM). Area under the curves (AUCs) for the two groups were: smokers, 2874±523 μU.min ml^{-1}; non-smokers 1534±168 μU.min ml^{-1}. Blood glucose began to fall at 5 min., reaching nadirs between 40-90 min. The pulmonary absorption of human insulin aerosol was ~ 75% for smokers and 25% for non-smokers assuming 100% absorption after subcutaneous injection. In Type I-diabetics insulin kinetics were similar to non-diabetics. Much more recently, Laube et al. (1993) at Johns Hopkins University investigated aerosolized insulin administration through the lungs in two normal subjects and six non-obese, non-insulin dependent diabetic subjects who had not received insulin prior to the study. A mean of 0.22U/kg regular U-500 insulin (Eli Lilly) was delivered by a jet nebulizer that generated aerosol into a holding chamber. Patients had to actuate 6, one second bursts of aerosol into the holding chamber and then inhale (to receive a 0.2 U/kg dose they repeated the procedure 8-13 times). Blood insulin peaked at 40±34 minutes. The average maximum decrease in plasma glucose from baseline was 55±10% vs 13±9% for placebo. They stated that the aerosol insulin was well tolerated, there were no side effects, and that aerosol insulin could be a reliable alternative to injection in controlling blood glucose in these subjects.

All human studies have indicated the feasibility of treatment of diabetes with aerosol insulin (Table 5). The peptide is 5.8 kD in size, bioavailability is good and aerosols appear to be well tolerated. In the three recent clinical studies, a modified nebulizer was connected to a special holding chamber which allowed specific numbers of breaths to be administered and quantified. Such devices enabled reproducible dosing and indicate the importance of good dosimetry if we are to advance the area of systemic delivery by aerosol. The Elliott (1987), Kohler (1987) and Laube (1993) studies each concluded that the variability in the glucose response from an aerosol was equivalent to that

TABLE 5

A Summary of Insulin Delivery Studies in Humans by Aerosol Inhalation

Investigators	Study Date	Number Doses[e]	Units/ Dose	Number of Patients	Insulin Type	Insulin T_{max} (Min)	Glucose T_{min} (Min)[c]	Absorption Relative to SC[d]
Gansslen	1925	1	30-50	5 Diabetics	Animal		30->120	
Wigley et al	1971	1	250	3 Normal, 4 Diabetic	Animal	15-60	30->200	
Elliott et al	1987	4-11	5-40	5 Normal, 6 Diabetic[a]	Human		30->200	20-25%
Kohler et al	1987	1-2	23±3	12 Normal[b]	Human	15	40-90	25-75%
Laube et al	1993	6	70±4	6 Diabetic	Human	40	153±27	20%

[a] Subjects were children - one took aerosol insulin for 6 months.
[b] 5 Smokers, 7 Non-smokers.
[c] Time at which blood glucose levels reached a minimum.
[d] Bioavailability relative to subcutaneous injection.
[e] Doses were administered in multiple inhalations in all cases.

seen by injection (±25%; Ziel et al., 1987). In humans, aerosol insulin seems to be more rapidly absorbed (peak at 5-60 min) and cleared from the blood than it is from the subcutaneous injection site (peak at 45-90 min; Guerra and Kitabchi, 1976; Berger et al., 1982; Murat and Slama, 1985; Edsberg et al., 1987). Kohler et al. (1987) found a mean serum peak of insulin at 15 minutes. In diabetics, however, clearance of regular insulin may be slower than in normals (Roy et al., 1980) because of circulating anti-insulin antibodies and thus, data from different studies should be compared carefully. More rapid glycemic control at meal times with aerosol insulin, in combination with once daily injections of long acting insulin, may even provide better control of the disease than is presently seen with injection. This, because regular insulin injected half an hour before a meal is absorbed too slowly to simulate the effect of insulin secreted endogenously in response to a meal (Ellenberg and Rifkin, 1983; Dimitriadis and Gerish, 1983).

Aerosol LHRH

The nonapeptide, leuprolide, is likely to be developed and released as an aerosol dosage form for oral inhalation in the treatment of female fertility disorders and prostatic cancer (Adjei et al., 1990; Adjei and Garren, 1990; Adjei and Carrigan, 1992). Despite its small molecular weight, it is a good illustration of a peptide where molecular modification has enabled delivery by aerosol. It is doubtful that leuprolide will be the only example of this kind. The compound is a synthetic nonapeptide and analog of naturally occurring gonadotropin releasing hormone (GnRH). It possesses greater biological activity than the normal compound which, when introduced systemically, induces leutinizing hormone and follicle stimulating hormone release from the pituitary (Schally et al., 1971). Pulsatile administration of GnRH analogs maintain pituitary function in GnRH deficient animals (Belchetz et al., 1978). These compounds can also be used, when plasma levels are maintained for longer durations, paradoxically, to desensitize the pituitary in hormonally sensitive conditions such as prostatic cancer and endometriosis (Leuprolide Study Group, 1984; Meldrum et al., 1982). The pharmaceutical chemistry story surrounding the development of leuprolide is instructive (Adjei, 1990). Because the naturally occurring decapeptide is refractory and chemically unstable, analogs were sought with better stability and increased activity. Other chemical modifications have centered on ease of systemic delivery via the pulmonary membranes. Two factors have been important. First, molecules have been synthesized containing groups which block the attachment of certain peptidases (Crooks and Damani, 1990). Second, smaller active molecules were also sought with larger absorption rates. The success of these approaches was shown in Adjei's data presented by Byron (1990c) and also in Adjei's publications (33-35, Table 4c). While the nasal bioavailability of leuprolide was poor, inhalation data was presented for different aerosol formulations. The aerosol suspension was considerably more bioavailable than the inhalation solution or the nasal formulations. The variability of the values for area under the curve following inhalation of leuprolide as a pressurized suspension metered dose inhaler was no greater than that seen after subcutaneous injection and is likely due to inter-individual variations in the intrinsic clearance of the compound (Byron and Clark, 1985). In another study in which the respirable fractions of the inhalation aerosols were estimated, Adjei and Garren (1990) have reported that leuprolide may be about 50% absorbed via the lung in humans when the material depositing in the back of the throat (Byron et al., 1989; Adjei and Carrigan, 1992) is subtracted from the apparent dose metered by the inhaler.

Absorption Enhancement

It is clear from Scheme 1 that the bioavailable fractions of all solutes administered to the lung may be enhanced by either increasing the absorption rate or decreasing the sum of the competing clearance rates. Niven and Byron (1990) and Hickey and Byron (1987) have shown that various surfactant molecules commonly employed in MDI formulations can enhance pulmonary membrane permeability to disodium fluorescein. While the magnitude of the changes in the dye's absorption rate was up to 3 fold, the required doses of surfactant were large. Also, absorption enhancement in the isolated rat lung, correlated well with an indicator of membrane toxicity, the induction of edema and loss of the preparation's viability (Niven and Byron, 1990). While the lung is subject to many

daily assaults and other, more sophisticated absorption enhancers are being tested using other routes of administration (Chien et al., 1989), a successful absorption promoter must cause only transient increases in membrane permeability and be non-irritating. Most importantly, it is likely, for pulmonary absorption, that promoters are not necessary (Table 4).

In the event that bioavailability is poor for a small compound (e.g., LHRH), one method of increasing the absorption rate constant, k_a, thus decreasing non-absorptive loss, is to increase K_D (Eq. 1). A more lipophilic analog may be better absorbed than its parent (Table 1). Alternately, a lipophilic prodrug (Kostenbauder and Sloneker, 1990) may be employed which is cleaved to the desired molecule after passage through the pulmonary epithelium. In the majority of cases however, the subject of absorption enhancement is left to the formulator who does not have the luxury of making changes in a drug's chemical structure. He or she may only choose the physical form(s) in which the chosen compound is administered alongside the substance(s) being co-administered. One method with the potential for enhancing K_D and thus, the absorption rate may be to employ an ion pairing approach (Florence and Attwood, 1988). In the event that a molecule carries a net charge, it may well be possible to formulate it with a counterion for which it possesses a high affinity in solution. In this case, the neutral complex may be expected to possess a larger value for apparent K_D than either of the individual ionized species at physiologic pH. Increasing apparent values for K_D may even work for macromolecules unable to pass through the tight junctions between epithelial cells. In order to explain the apparent transfer of macromolecules at finite rates it seems necessary to propose a phenomenon like endocytosis. This may act both to internalize macromolecules within epithelial cells and also to transfer them into the interstitium between epi- and endothelia. The pulmonary endothelium is known to be more permeable to large molecules like albumin than is the epithelium (Byron and Phillips, 1990). Hubbard et al (1989) have also shown that large proteins like alpha-1-antitrypsin can be preferentially drained into the pulmonary lymphatics and from there into blood, rather than passing directly from airway to circulation. Endocytosis of this kind would also be expected to proceed down a concentration gradient, but more importantly, improving the lipophilicity of macromolecules like PHEA has been shown to enhance endocytosis. Duncan et al (1982) covalently linked hydrophobic tyramine to the otherwise hydrophilic polymer, PHEA, and enhanced the rate of endocytosis. "Adsorptive pinocytosis" was proposed as an alternative to "fluid phase pinocytosis" due to the increased affinity of the hydrophobic macromolecule for the cell membrane. In the event of active transport occurring (Fig. 3), it may be possible to make structural modifications which favor an analog more than the original drug. In these cases the transport is likely to be saturable and therefore, the bioavailable fraction should increase as a function of decreasing dose. The structural dependence of such transport processes in the lung is presently unknown.

Regional Deposition

Other subjects which presently remain to be studied concern the manipulation of other variables in equation 1. While the overall thickness of the absorption barrier, h, must decrease as the respiratory tract is descended, it is unlikely that we can presently achieve such control over aerosol particle size and breathing regime that the site of drug deposition is constrained to say, the alveoli alone. Drug administration and release in different regions of the GIT often result in altered absorption kinetics, due to large differences in pH and surface area for absorption between regions. Fortuitously, this may not be a significant problem for inhalation aerosols containing small molecules, even though drug may be deposited over a small or a large surface area. As shown in equation 1, k_a should be dependent on the ratio of surface area to fluid volume in the airways, A_M/V. Appropriate values for A_M and V are debatable. However, it is interesting to observe that solution aerosol delivery in the rat lung only achieves a two-fold enhancement of k_a over intratracheal instillation (small molecules; Schanker, and Byron and Phillips, 1990). Instillation is believed to produce drug deposition over a much smaller and more central area of the lung in which the values for h (equation 1) are somewhat larger. Similarly, solid fluorescein aerosols administered to the dog lung showed no difference in k_a when the aerosol sizes were increased from 1 to about 4.5 μm despite probable differences in their sites of deposition (Clark and Byron, 1985). Several discussions

have appeared in the literature (Byron and Phillips, 1990; Effros and Mason, 1983; Clark and Byron, 1985; Brown and Schanker, 1983) and it is important to note that, for small molecules, solute concentration in the airways does not appear in equation 1. This is important because an exponential increase in surface area occurs as the respiratory tract is descended (Gerrity, 1990). Thus, for a given dose, an exponential decline in solute concentration in the airways is expected to result from aerosol deposition progressing further toward the lung periphery. In reality, for a passive absorption process, k_a should be independent of these concentrations, and only affected by the ratio $A_M/(Vh)$; D may be considered constant for a chosen solute (equation 1). Fortuitously, as the area for drug deposition is increased, so is the volume of fluid lining the airways. The net effect, for the extremely small volumes of aerosol usually employed, is to defy a commonly stated assumption that drug depositing lower in the respiratory tract must be absorbed orders of magnitude faster, just because of the massive surface area for absorption in the lower lung. From a practical pharmaceutical perspective, minor variations in aerosol size and sites of deposition are not likely to cause significant variations in drug absorption kinetics for small molecules. Thus, the important conclusion is that minor changes in deposition are unlikely to cause large changes in k_a from small metered doses. This observation is consistent with the apparent independence of cromolyn sodium's absorption in humans after substantially different drug deposition patterns were induced with and without a methacholine bronchoconstrictive challenge (Richards et al., 1988). This however, is a small molecule which is absorbed in rats rapidly and actively (Gardiner and Schanker, 1974) and a similar argument may not be true for macromolecular compounds absorbed by different mechanisms (Fig. 2). Many of the proteins and peptides are hydrophilic and they may pass to some extent through epithelial tight junctions. As their size increases however, endocytosis probably plays an increasing role (Wangensteen, 1991). Animal data for leuprolide in dogs (Adjei et al., 1990) and insulin in rabbits (Colthorpe et al., 1992) indicates that the bioavailable fraction of both compounds decreases substantially as the compound is deposited higher in the respiratory tract. Thus, the site of deposition of the aerosol may become an extremely important determinant of a macromolecule's bioavailable fraction. This may be because of regional differences in pulmonary permeability (k_a) or metabolism. The effect may also be enhanced with molecules possessing decreased absorption rates. In these cases, the rapid mucociliary clearance component associated with the upper airways may well have increased significance and decrease bioavailability considerably.

CONCLUSION

Small peptides and proteins ($\leq$ 20-30 kD) can feasibly be administered via the lung as aerosols for systemic therapeutic purposes provided systemically required doses are no more than a few mg. The route may also be possible for larger molecules if some sacrifices in bioavailable fraction are acceptable. Bioavailable fractions of important biotechnologic products appear, in many cases, to be large and reproducible in animal and human studies reported to date. Although absorption rate constants have often been reproducible and independent of regional deposition for small molecules, this cannot always be assumed to be true for proteins and macromolecules. Bioavailability for hydrophilic polypeptides may well prove to be dependent upon their regional deposition in the lung. Accordingly, inhalation systems are required for efficient reproducible aerosol delivery of macromolecules. The pharmacologic and toxicologic consequences of chronic macromolecule delivery by inhalation also requires further study.

APPENDIX - POSTER SYMPOSIUM SUMMARY

This symposium consisted of 12 posters (*J. Aerosol Med.* (1993), 6 Suppl. 51-55) which dealt with peptide and protein absorption (6 posters), small molecule systemic effects (3 posters), methods to control the release of aerosol drugs (2 posters) and the toxicity of pentamidine analogs to lung cells (1 poster). Of the six posters on the systemic delivery of peptides and proteins, two dealt with proteins and peptides, three with analogs of the small 9 amino acid peptide, luteinizing hormone

releasing hormone (LHRH), and one examined the absorption of synthetic polyasparagines. The permeability of the lung to peptides and proteins is very high compared to other mucosal surfaces (i.e. gastrointestinal, nasal etc.) and there is growing interest in this route for the systemic delivery of therapeutic agents that would otherwise have to be injected.

Dr. Patton (Palo Alto, USA) gave a summary of the systemic bioavailabilities of a variety of clinically relevant peptides and proteins as measured by the rat intratracheal (IT) technique. Although not all peptides and proteins covered in the poster were absorbed, the majority of compounds gave bioavailabilities of 20-95%. So far, this simple technique appears to be a representative model for determining the permeability of the mammalian lung to macromolecules. They emphasized, however, that with regard to efficiency of delivery to the lung, injection of liquid material into an animal's lung is ideal, being nearly 100%, while current aerosol devices for delivering to humans are only 10-20% efficient.

There were questions about the effect on protein absorption of active water absorption (Pritchard, London, UK) and the heterogeneity of lung distribution resulting from the IT technique. Patton stated that IT and aerosol delivery could show similar overall rates of absorption but that the results may not necessarily concur. For example, growth hormone is more slowly absorbed by IT than by aerosol. Shanker and colleagues have shown in a series of papers that rates of absorption following aerosol administration are usually about double that seen with IT delivery. Niven (Thousand Oaks, USA) made the point that the vehicle could also influence pulmonary absorption. Williams (Boston, USA) asked if some low molecular weight peptides are broken down or just not available. Patton said that with radioimmunoassays it was difficult to say.

In work that pointedly underscored one of the key issues in the field, Colthorpe et al. (Cardiff, GB) presented a poster on pulmonary absorption of insulin and oxytocin in rabbits in which the kinetics of aerosol and intratracheal absorption were compared. Bioavailability of both peptides was markedly better from an aerosol (57-68%) than from intratracheal instillation (6-9%), which was not consistent with their predicted alveolar deposition values (73% for aerosol and 46% for intratracheal). It was not clear why the aerosol was so much better than intratracheal but as Adjei and Carrigan (1992) have shown (*J. Biopharm Sci.* 3:246-254) depth of deposition of a peptide solution into the lungs can dramatically effect the peptides bioavailability. Volume of instillate is also important. Byron (Richmond, USA) asked how k_{lb} and k_{le} were determined. Dr. Colthorpe said that k_{lb} was assumed to be given by the product of bioavailable fraction, F x k_a; that is, that unabsorbed drug was assumed to be eliminated from the lung according to the value of k_{le}.

The first two posters on the LHRH analogs were presented by Dr. Adjei (Chicago, USA) who summarized the large body of work conducted at Abbott over the last decade on leuprolide, an LHRH analog. Because leuprolide is blocked at both the N and C terminus of the peptide it is stable to lung peptidases. In dogs, deposition of the peptide as a solution at the bifurcation of the trachea led to quantitative systemic absorption. In humans, bioavailabilities from a metered dose inhaler (MDI) relative to subcutaneous delivery (which included device losses) were 7-28% and depended on the formulation. The second poster presented results from a 14-day, repeat dose, aerosol study in dogs in which a linear dose response was observed over the course of the two-week study and no toxicological effects were observed. Teitelbaum (Palo Alto, USA) asked if the AUCs were the same for both formulations at day 1 and 14. Dr. Adjei said they were.

Dr. Teitelbaum (Palo Alto, USA) then presented his work carried out at Syntex on detirelex (DTRX), a hydrophobic decapeptide LHRH analog. They examined the systemic absorption of both solution and liposomal formulations in the dog. Unlike Abbott's leuprolide which exhibited peak serum levels at 30-60 minutes after delivery, DTRX in solution showed much slower absorption with peak serum levels at 6-12 hrs (and a bioavailability of 30% relative to IV). Following encapsulation in negatively charged liposomes, lung absorption was even further prolonged to give serum maxima between 32-48 hrs. Patton (Palo Alto, USA) pointed out that taken together with the Abbott findings, the Teitelbaum results were promising for pulmonary peptide development because they demonstrated that the absorption profile of a peptide could be tailored by chemistry as well as formulation to be rapidly or slowly absorbed from the lung.

Dr. Rypacek (Prague, Czech Republic) presented a poster on the dose, charge and molecular size dependence of cationic polyasparagine (47-54 amino acids, MW weight averages 7.7-8.6 kD)

absorption from the isolated perfused rat lung which can be used to study absorption for about 3 hours. The kinetics of polymer absorption were studied as a function of time (0-3 hr), dose (0.2-5 mg) and polymer charge (positive or neutral). No charge effect was seen. Although absorption rates increased with dose and were maximal at the highest dose, at the high dose (5 mg) 9.6% was absorbed in 3 hr while 34.4% of a 0.2 mg dose was absorbed in the same period. There was some molecular sieving during absorption such that when a mixture was placed into the lung with an average molecular weight of 8 kD, the average weight of the mixture recovered in the perfusate was reduced to 6.7 kD. Gonda (South San Francisco, USA) wondered why there was no charge effect. Rypacek said it was very strange and he did not know why.

In another application of systemic therapeutic delivery, Carpin et al. (Aberdeen Proving Ground, USA) presented a poster on the aerosol delivery of an opioid agonist/antagonist "cocktail" in the rat. The idea was that by delivering the two opposing agents together, that the negative side effects of the agonist could be counteracted by the antagonist without completely sacrificing agonist efficacy. Rubesaman (Heyward, USA) found the approach hard to accept and stated that in the field of anesthesia it is very difficult to calculate dose, that dose levels were usually elevated until the right effect was achieved, and that antidotes could be used if an adverse effect occurred. Dr. Carpin agreed that doses were hard to calculate and said that rats and ferrets were sensitive to different ratios and cocktails. He noted that the agonist alone was normally fatal to ferrets but this was not the case in the presence of antagonist. He also stated that their approach was more appropriate for the field situation than for the well-staffed and equipped hospital.

There were two posters presented on the manufacture of solid particulate aerosols designed to release drug slowly following their delivery via inhalation. The philosophy was to retard the release of locally acting drugs in the respiratory tract which would otherwise be absorbed rapidly and lost from the site of action. Haghpanah, et al. (London, GB) presented a method for preparation of heat denatured albumin microspheres. The spheres were prepared in the absence of surfactant by emulsification and heating. Particle sizes (measured microscopically and aerodynamically) were related to their respective method of manufacture. Significant fractions were respirable as assessed by twin stage impinger experiments. Discussion of the paper centered around likely toxicities which might be expected as a result of chronic administration of denatured proteins by inhalation. Dr. Yeates (Chicago, USA) said that similar preparations were currently approved by the FDA for use in nuclear medicine and that adverse responses have not been a problem following one or two inhalations. Yeates et al. (Chicago, USA) had developed a technique of spray drying a highly water soluble compound, disodium flourescein, concentrating it, and condensing the vapor phase onto the dry particulate surface of paraffin wax or lauric acid, using the dry particles as nuclei. Resultant coated particles were less than 5 μm in diameter, showed differences in release kinetics as plasma fluorescein concentration vs. time profiles, when administered to beagles by inhalation. The time at which maximum plasma concentration occurred was inversely related to the rate of release in vitro. Discussion centered on the likely fate of the wax. Although paraffin is relatively inert, it has been known to cause serious reactions when implanted subcutaneously for cosmetic reasons and the fact that waxes are known to reside in the lung for long periods of time was thought to be a problem. Disodium fluorescein is believed to be insoluble in paraffin wax and the mechanism of its release was unknown. Different wax to disodium fluorescein ratios were achieved by increasing the temperature of paraffin wax in the Sinclair-La Mer generator. Dr. Patton (Palo Alto, USA), emphasized the importance of developing carriers which did not accumulate during chronic administration of aerosols to the lung. He believed that this was the only biocompatible way of making sustained release pulmonary systems for long term use and chronic administration to patients.

Three posters were concerned with toxicity following aerosol administration. Frank-Piskorska et al. (Warsaw, Poland) had studied bone mass density using single photon absorptiometry in 20 human asthmatics treated with budesonide (standard dose: 800 μg/day for 6 months). Despite reports of alterations of the steady state between bone formation and bone resorption following high dose steroid administration, this technique was not sufficiently precise to detect changes over the 6-month trial period involving standard dose administration. Discussions centered around the need to increase patient number and trial durations in order to begin to quantify the reported effects. Hansen and Andersen (Odense, Denmark) had studied the pharmacodynamics of the cardiovascular side effects

due to high dose salbutamol (albuterol) inhalations. Even though they failed to collect data on serum potassium, they reported that systemic effects on pulse rate and arterial saturation occurred too quickly following inhalation for these effects to be attributed to the swallowed portion of the drug. This conclusion was contrary to a literature report which implied that some of these systemic effects can be due to oral absorption after MDI dosing.

The final paper in this section by Farr et al. (Cardiff, GB and Chapel Hill, USA), involved local, not systemic toxicity. In some elegant experiments on freshly isolated and 24-hour cultured alveolar type II cells, the authors studied differential toxicity of pentamidine, four of its metabolites and five pentamidine analogs. Changes in cell adhesion were used as an index of toxicity. Pentamidine was readily accumulated by type II and type I cells (during cultivation, type II cells differentiate to form type I cells). Toxicity could be correlated with increasing hydrophobicity and increasing basicity. C-hydroxylated metabolites were fairly toxic while N-hydroxylated metabolites were exceptionally toxic. This latter observation was contrary to the situation found in the liver. Discussion centered on the mechanism of toxicity. It was neither known whether toxicity was related to antimicrobial effects, nor was there any information currently on the differential toxicities when compounds were applied to different cell types in the lung. In addition, it was not known if the epithelium was able to recover from pentamidine induced damage. Dr. Williams (Boston, USA) stated that there was some evidence in certain cases that the alveolar stem cells could be killed.

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