Solute Absorption from the Airways of the Isolated Rat Lung. V. Charge Effects on the Absorption of Copolymers of *N*(2-hydroxyethyl)-DL-Aspartamide with DL-Aspartic Acid or Dimethylaminopropyl-DL-Aspartamide

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Purpose. To determine the effects of ionized substituents upon the pulmonary absorption of 6–8 kDa synthetic, hydrophilic polypeptides.
Methods. Fluorophore-labeled poly (hydroxyethylaspartamide), F-PHEA (neutral at pH 7.4) and its copolymer derivatives poly (hydroxyethylaspartamide-co-dimethylaminopropylaspartamide), F-P(HEA-DMAPA) (positive at pH 7.4) and poly (hydroxyethylaspartamide-co-aspartic acid), F-P(HEA-AA) (negative at pH 7.4) were synthesized and administered in different concentrations to the airways of the isolated rat lung preparation. The time and molecular weight dependencies of polypeptide absorption into perfusate were determined at intervals by gel permeation chromatography.
Results. For all polypeptides, molecular weights in perfusate were about 1 kDa less than those which were administered, due to preferential absorption of smaller molecules. The absorption, up to 70% of the administered dose over 3 h, of the anionic F-P(HEA-AA), was significantly faster than that of the neutral F-PHEA or the polycationic F-P(HEA-DMAPA). The latter derivative produced greatest edema in the lung. Absorption showed both active [dose-dependent kinetics] and passive [diffusive] components for all three polymers.
Conclusions. Pulmonary absorption of similarly sized macromolecular PHEA derivatives, either neutral, positively or negatively charged, occured via carrier-mediated and diffusive mechanisms. The highest rate of absorption was observed with the polyanionic derivative.

KEY WORDS: pulmonary absorption; isolated rat lung; polypeptides; macromolecules; solute ionization.

INTRODUCTION

Although the lungs are unusually effective as an absorption site for macromolecular xenobiotics (1), transport mechanisms and their molecular dependence are not well characterized. In this paper, we continue to explore the nature of macromolecular transport through the epithelia of the lower lung. Our last paper in this series (2) described and characterized the pulmonary absorption of a fluorophore-labeled, model polypeptide, poly-α,β-[N (2-hydroxyethyl)-DL-aspartamide], F-PHEA, which was polydisperse with respect to molecular weight. Its absorption kinetics were described by the sum of an active, dose-dependent process (F-PHEA transporter; evident over the first 30 minutes of airway-to-perfusate transfer) and simultaneous diffusion (which occurred throughout each 180 minute experiment). The F-PHEA transporter was subject to competition from fluorophore-free, neutral PHEA with otherwise identical molecular characteristics (2). This paper compares the dose-dependency of the airway-to-perfusate transfer kinetics of two F-PHEA derivatives, to that of F-PHEA itself. The derivatives carried different, multiple electronic charges, due to their existence in pH 7.4 aqueous solution as polyanions or polycations. Their molecular weight distributions, MWD, and hydrodynamic radii, R_h, in solution, were matched to that of a new batch of F-PHEA to which they were compared. Because of literature reports, which describe the presence of ionized loci within the airways (3–5), we hypothesized that the charge and dose dependency of macromolecular pulmonary absorption may change as a function of solute ionization, and that a comparison may illuminate the nature and location of the F-PHEA transporter in the alveolar regions of the lung.

MATERIALS AND METHODS

Synthesis and Characterization of F-PHEA, F-P(HEA-DMAPA) and F-P(HEA-AA)

Preparation of fluorophore-labeled poly-α,β-[N (2-hydroxyethyl) D,L-aspartamide], or F-PHEA, was described previously (2). Briefly, polysuccinimide (PSI) was prepared by thermal polycondensation of aspartic acid and reacted with 2-aminoethylcarbonyl-6-aminofluorescein (to give F-PSI), followed by reaction with excess ethanolamine, to produce F-PHEA. The F-PHEA derivatives containing either N,N'-dimethylaminopropyl side chains, random co-polymer poly α,β (HEA-co-dimethylaminopropyl aspartamide), or carboxylic acid side chains, random copolymer poly α,β (HEA-co-aspartic acid), were produced by initial reaction of F-PSI with stoichiometrically controlled amounts of the bases, 3-dimethylaminopropylamine or sodium hydroxide, respectively, prior to completion of aminolysis of F-PSI by excess ethanolamine (Fig. 1). All of these hydrophilic polymers were purified by repeated gel filtration in 0.1 M NaCl [Sephadex G25, Pharmacia, Uppsala, Sweden], followed by exhaustive dialysis with water and lyophilization. Each polymer was characterized in terms of its molecular weight distribution (MWD) and fluorophore content (6). The content of ionizing substituent groups and the absence of counterions was determined by potentiometric titration (in water) with NaOH or conductiometric titration (in isopropanol-water) with sodium propanolate. Synthetic techniques were optimized to ensure that the hydrodynamic volume distribution of the co-polymers were comparable to that of F-PHEA, as determined by similar elution characteristics following high performance gel permeation chromatography (6). To ensure that

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ABBREVIATIONS: DMAPA, *N,N'*-dimethyl-aminopropyl-D,L-aspartamide; AA, aspartic acid; F-PHEA, fluorophore-labeled poly-α,β-[N-(2-hydroxyethyl)-D, L-aspartamide]; IPRL, isolated perfused rat lung; M_n, number average molecular weight; M_w, weight average molecular weight; MWD, molecular weight distribution; R_h, hydrodynamic radius; Q_d, concentration normalized diffusion quotient.

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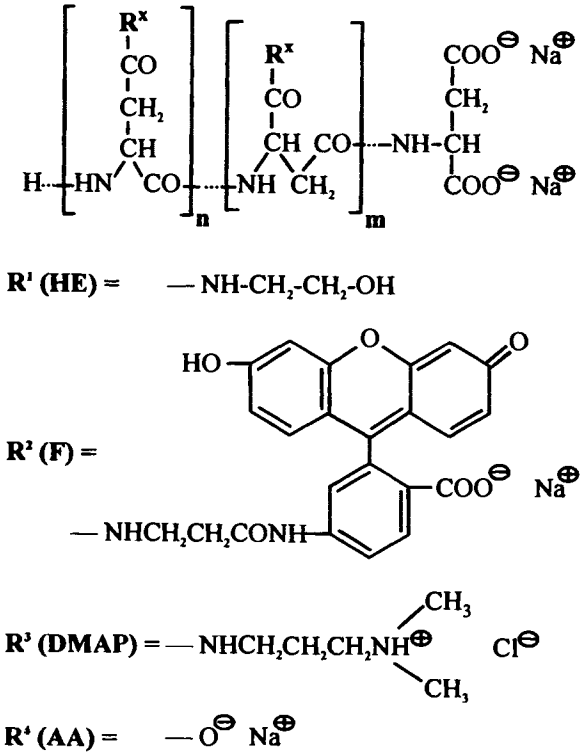

Fig. 1. Structure of polymers. F-PHEA and its copolymer derivatives are constituted of a random combination of α- and β-peptide bound aspartic acid units (n ≃ m) with side chains R^x (x = 1–4) as shown. The composition of the side chains R^1–R^4 for each polymer is given in Table 1. Electronic charges are shown as they are assumed to be, following dissolution in 0.15M NaCl, pH 7.4.

diffusivity of polymers was not affected by either molecular aggregation in solution, or significant increases in solution viscosity with increased polymer concentration, diffusion quotients [proportional to aqueous diffusion coefficients] were determined for each of the polymers, at pH 7.4 and 37°C, with donor phase concentrations ranging across those employed for dosing the airways, using the rotating diffusion cell technique and materials reported previously (2).

Pulmonary Absorption

Aqueous solutions of each polymer [F-PHEA, F-P(HEA-DMAPA) and F-P(HEA-AA)] with different concentrations (2, 10 and 50 mg/ml) were prepared and adjusted to pH 7.4 with HCl or NaOH. The IPRL preparation and the dosing method are carefully controlled in these laboratories and have been described in detail previously (7). These were unchanged and animal research adhered to the NIH Principles of Laboratory Animal Care. Briefly, a rat lung was surgically removed and housed in an artificial thorax maintained at 37°C. Krebs-Henseleit solution with 4% (w/v) bovine serum albumin was used as perfusate (300 mL) and recirculated through the pulmonary circulation via the pulmonary artery at a constant flow rate of 15 mL/min. A metal dosing cartridge containing 0.1 mL polymer dosing solution was inserted into the trachea via a tracheal cannula. A metered dose inhaler (containing propellants only) was connected to the dosing cartridge and actuated once. The dosing solution was propelled into the lung as a coarse spray, and the lung was inflated simultaneously to about 6 mL. The dosing cartridge was removed and the lung allowed to deflate. The perfusate samples were taken from a well mixed reservoir at time 0 (blank sample, immediately prior to dosing) and then subsequently at 15, 30, 60, 90, 120, 140, 160 and 180 min following dosing. Sufficient IPRL preparations were studied to yield four fully viable preparations for each dosing solution, as evidenced by the absence of any signs of edema onset over 180 min [we have shown previously and observe consistently with this preparation, that "signs of edema onset" occur when the (blood-free) lungs change in outside color and texture from smooth white to a grey and/or patchy appearance (8). Changes which occur subsequent to these early indicators of the preparation's declining viability, such as discontinuous increases in epithelial permeability causing increasing airway-to-perfusate transfer of solutes, increasing wet lung/dry lung ratios and other effects (8,9) were excluded from this discussion by discarding such preparations.] Polymer concentrations in perfusate were determined by high performance gel permeation chromatography as described previously (HPGPC; 10). Chromatograms were also analyzed for molecular weight distribution (MWD) of polymer in perfusate (2,6,11).

Data Analysis

Statistical analysis was performed throughout by using analysis of variance (ANOVA) and Student's t-test. Single factor ANOVA was used to test the hypothesis that means from several samples were equal. Two tailed, non-paired Student's t-test was used to test whether two sample means were equal. Alpha was set at 0.05.

RESULTS AND DISCUSSION

Characterization of F-PHEA, F-P(HEA-DMAPA) and F-P(HEA-AA)

Table 1 details the major characteristics of the polymers used in this study. Although differences existed between polymers in apparent M_w and M_n [due to their different syntheses], the mean GPC elution curves (n = 3) for F-P(HEA-DMAPA) and F-P(HEA-AA) fell within and occupied > 85% of the area under the mean GPC elution curves for F-PHEA itself, showing that each polymer had a similar distribution of hydrodynamic radii in solution (12). Table 1 also shows the content of fluorophore, ionizable groups and the hydrophilic, neutral, hydroxyethyl substituent. Titration-derived pKa values (Table 1) for DMAPA and AA substituents showed that, at pH 7.4, co-polymers containing these substituents were polycations or polyanions due to N-protonation or deprotonation of carboxylic groups, respectively. Diffusion quotients for all polymers (Table 1), through 50kDa cutoff dialysis tubing (proportional to aqueous diffusivity; 2) were independent of concentration over the range of concentrations employed during dosing, showing that the diffusing species and their aqueous diffusivities were apparently independent of concentration in the dosing solutions.

Pulmonary Absorption of F-PHEA, F-P(HEA-DMAPA) and F-P(HEA-AA)

This work is based upon studies in an isolated perfused lung preparation in which only the pulmonary circulation is

Table 1. Polymer Characteristics

Polymer	[R[1]	R[2]	R[3]	R[4]][a]	M_w[b]	M_n[b]	Pd[c]	DQ[e]
F-PHEA	99.7	0.3	0.0	0.0	7420 (220)	6620 (180)	1.12	5.17 (.19)
F-P(HEA-DMAPA)[d]	87.7	0.3	12.0	0.0	6490 (420)	6000 (340)	1.08	4.19 (.37)
F-P(HEA-AA)[d]	83.4	0.3	0.0	16.3	8090 (300)	7340 (280)	1.10	1.11 (.16)

[a] Mole % monomer units carrying substituent; see Figure 1.
[b] M_w and M_n are HPGPC-determined weight and number mean molecular weights (SD; n = 5), respectively, based upon column calibration using F-PHEA standards.
[c] Pd = M_w/M_n.
[d] Polymers were >95% free of counterions; AA and DMAPA pKa values were 3.8 and 9.25, respectively.
[e] Diffusion quotient (SD; n = 9) = (steady state flux)/(donor phase concentration) in μL/h) at 37°C and pH 7.4; proportional to aqueous diffusion coefficient. No concentration dependence was observed in the range 2 through 50 mg ml^{-1}.

perfused (the bronchial circulation is severed during preparation; 8). Dissolved solute delivery to the airways has been optimized previously (7). In control experiments, polymer delivery was shown to be reproducible (consistently > 80% of the administered dose to the lobar regions, from which polymer absorption can be expected to occur), and independent of polymer concentration in dosing solutions of F-PHEA itself (2), and its co-polymers. In our previous studies of F-PHEA airway-to-perfusate transfer (2,6), absorption was described by the sum of an active, dose-dependent process (F-PHEA transporter), which was most evident in the first 30 minutes following administration, and simultaneous diffusion, which continued throughout the lifetime of the preparation. The F-PHEA transporter was subject to competition from fluorophore-free, neutral PHEA with otherwise identical molecular characteristics; PHEA significantly suppressed the % transfer of small doses of F-PHEA over the first 30 minutes (2). Similarly, recent additional experiments, to be published in full later, have shown that the presence of 200 μM ouabain (an intracellular ATP-to-ADP conversion inhibitor; 13) in perfusate, caused significant suppression of F-PHEA transport. In control experiments in the present study, where polymers were added to perfusate in the presence and absence of the IPRL, there was no evidence of fluorophore release, polymer metabolism or binding losses to the apparatus. Table 2 shows the nominal and the mean administered dose to the airways, for each of the polymers, alongside the actual number of IPRL preparations which were studied, in order to yield four viable, non-edematous preparations (larger numbers were required when polymer toxicity induced early signs of edema in the lung). Using these data as an index of toxicity in the airways, polymer toxicity ranked F-P (HEA-DMAPA) > F-P(HEA-AA) > F-PHEA at the highest administered doses. This was consistent with literature reports of enhanced membrane toxicities for polycations (14–16).

IPRL preparations were defined as viable, when they showed no evidence of lung edema (8; see definition in methods and note that the term "viability" does not imply or require functioning active transport systems for the entire experimental duration) for these 180 minute experiments; absorption data from non viable preparations was discarded. [This population selection process, which is similar to many used in whole animal procedures, is applied uniformly to all of our IPRL studies (a) because preparative and dosing aberrations themselves can induce early edema/transport anomalies (9) and (b) the purpose of the present study was to compare transfer kinetics between polymers in intact "viable" preparations.] Percentage transfer to perfusate, with respect to administered dose, is shown as a function of time, for each polymer-dose combination in Fig. 2. Differences in the absorption rates of different polymers were most pronounced for the smallest dose, small to insignificant for medium dose and absent for the largest dose. As we have observed previously for neutral F-PHEA of different molecular weights (2,6), the most rapid absorption (in the first 30 minutes), for all of these polymers, was clearly dose and/or dosing solution concentration dependent (final column, Table 2). Although, in the case of F-P (HEA-AA), there was some evidence that this dose-dependency was extended beyond 30 minutes, this was difficult to substantiate. The early, dose-dependent transfer to perfusate was followed (t = 30 min through 180 min) by apparent passive absorption thereafter, in which the average %

Table 2. Polymer Dosing, IPRL Viability and Percent Transfer to Perfusate at 30 min.

Polymer	Dose [mg ± SD] Nominal	Dose [mg ± SD] Administered	IPRL number to yield n = 4	Percent administered dose in perfusate at 30 min.
F-PHEA	0.2	0.19 ± 0.01	4	31.5 ± 17.4
	1	0.85 ± 0.09	4	15.6 ± 3.3
	5	4.39 ± 0.26	6	9.2 ± 3.2
F-P(HEA-DMAPA)	0.2	0.17 ± 0.02	9	18.6 ± 0.9
	1	0.83 ± 0.09	7	15.1 ± 3.9
	5	3.77 ± 0.58	14	6.4 ± 2.7
F-P(HEA-AA)	0.2	0.18 ± 0.01	4	38.8 ± 15.9
	1	0.84 ± 0.06	7	23.9 ± 11.2
	5	3.67 ± 0.48	8	6.9 ± 2.6

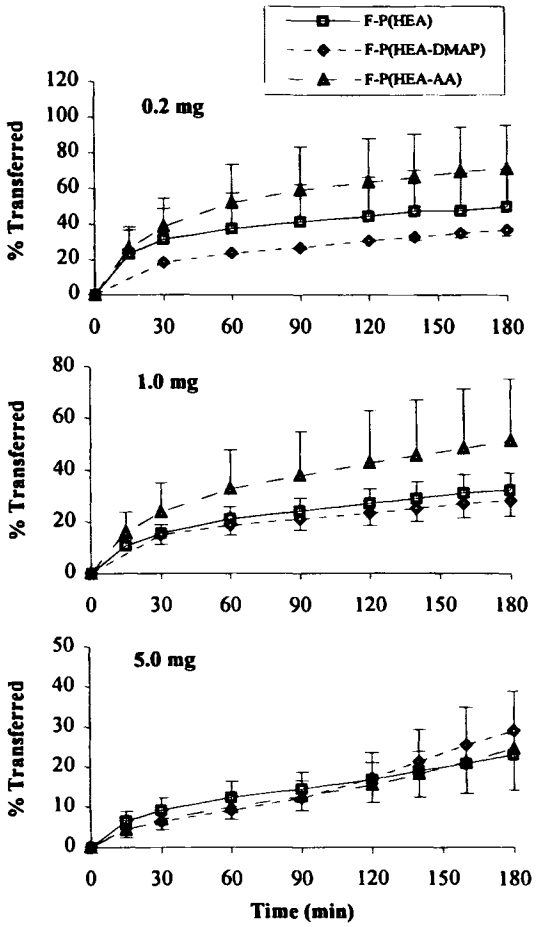

Fig. 2. Mean percentage of administered doses in perfusate vs time. Error bars are standard deviations [n = 4]. Results are grouped by nominal dose [0.2, 1.0 and 5.0 mg in upper, middle and lower panels, respectively [see Table 1]. Note that the scale of the y-axis is expanded in the lower panels.

transfer rate (% per minute), was largely dose-independent (Table 3). Between-dose differences in % transfer rates, within polymers, were statistically insignificant over this time period [average slopes from linear regression (n = 4 per dose) were compared using ANOVA; Table 3]. However, the average transfer rates across doses during this 30 to 180 minute period [average slopes from linear regression of % transfer data (n = 12 per polymer)], differed significantly between polymers, and ranked F-P(HEA-AA) > F-P(HEA-DMAPA) > F-PHEA with values of 0.165 ± 0.074, 0.121 ± 0.048 and 0.106 ± 0.024% min^{-1}, respectively ($p < 0.05$, ANOVA). Comparing the pulmonary absorption of these polymers at the same nominal doses (Fig. 2) showed, contrary to conventional wisdom, that negatively charged F-P(HEA-AA) showed significantly larger transfer rates in the 0.2 mg and 1 mg nominal dose groups, when compared with F-PHEA or F-P(HEA-DMAPA). An average of 71% of the nominally 0.2mg administered dose (actual values in Table 1) of F-P(HEA-AA) was absorbed in 3 hours, vs 50% for F-PHEA and 37% for F-P(HEA-DMAPA). At the 5 mg nominal dose level, as dose-dependent, carrier-mediated transport tended toward saturation, the three different polyaspartamides had similar values for percentage absorbed after 3 hours. At lower doses however, the polyanion, F-P(HEA-AA), was absorbed at similar or greater initial rates ($t \leq 30$ min), and greater final rates (30 min $\leq t \leq$ 180 min), than either the neutral or the cationic polymer.

Table 3. Average Airway-to-Perfusate Polymer Transfer Rates [%·min^{-1} ± Standard Deviation] 30 to 180 min. After Airway Administration, Showing a Lack of Dose-Dependency in this Absorption Phase

Nominal dose [mg]	F-PHEA	F-P(HEA-DMAPA)	F-P(HEA-AA)
0.2	0.120 ± 0.024	0.121 ± 0.029	0.203 ± 0.054
1.0	0.108 ± 0.026	0.087 ± 0.026	0.177 ± 0.086
5.0	0.090 ± 0.016	0.155 ± 0.061	0.115 ± 0.068

The "molecular weight sieving effect," reported previously for F-PHEA with weight mean molecular weights greater than approximately 8 kDa [6], occurred with all of these polymers independent of their charge. As before, smaller molecules were absorbed preferentially, although changes in MWD over time and dose were not statistically significant. Weight averaged molecular weights of absorbed polymers were determined by HPGPC, on a column calibrated with F-PHEA standards, and found to be up to 1 kDa smaller than those which were administered (Table 1). Values at 120 minutes (expressed as if each polymer was F-PHEA) were 6.37 ± 0.21 kDa [F-PHEA], 6.08 + 0.26 kDa [F-P(HEA-DMAPA)] and 7.40 + 0.24 kDa [F-P(HEA-AA)], respectively. Small reductions in polydispersity also occurred due to absorption. Starting values for Pd = M_w/M_n are shown in Table 1; at 120 min following absorption, these became 1.09 [F-PHEA], 1.07 [F-P(HEA-DMAPA)] and 1.09 [F-P(HEA-AA)], respectively. When the hydrodynamic radii, R_h, of the absorbed polymers were calculated (12), R_h ranged between 1.6 and 1.8 nm. Values were much larger than those calculated for equivalent pore radii in lung epithelium (0.6 to 1.0 nm; 17), and implied that passive transfer through epithelial tight junctions alone, could not explain the extent of their absorption.

The last paper in this series (2), discussed a "multiple mechanism" type of absorption for F-PHEA, a significant proportion of which was transported by endocytosis, the proportion increasing, as doses were decreased. PHEA (fluorophore-free) was shown to compete with F-PHEA for its transporter (2), eliminating the possibility that transport was due to the label itself. The presence of airway-to-blood transporters for small anions has been noted previously (18,19) and the literature documenting active re-uptake of multiply ionized albumin from the alveolar spaces is expanding (20 and references therein). While it is possible that each of the polymers tested in the present study has a separate transporter, it is thus tempting to suggest the presence of a rather non-specific carrier in the perfused lower airways of this preparation. Such a carrier could endocytose macromolecules up to a certain size, with little specificity to their charge. Although this would explain the broadly similar dose dependencies for each of these polymers (Fig. 2), it also suggests that the transporter's location is the alveolar type I cell. This thin flattened cell comprises the bulk of the gas exchange surface of the lung and, unlike the alveolar type II and endothelial cell surface, appears to lack anionic sites on its luminal surface (4). The presence of such a non

specific transporter may explain the diversity of macromolecules which are found to cross the pulmonary barrier and support the implication of "caveolae" in such uptake processes (1).

CONCLUSIONS

The absorption of three differently charged, but otherwise similar polymers were compared at different dose and concentrations in non-edematous IPRL preparations. Polycationic F-P(HEA-DMAPA) was least well tolerated by the preparation from the point of view of its tendency to induce lung edema. Two phases were apparent in the absorption process for all polymers. During the initial phase ($t \leq 30$ min), a dose-dependent process prevailed, indicating the presence of one or more endocytic macromolecule transporters in the lower airways which functioned similarly, irrespective of polypeptide electronic charge. After the initial period, transport continued by dose-independent mechanisms. Overall, polyanionic F-P(HEA-AA) appeared to be transported preferentially and, if a single PHEA transporter was assumed, the data suggested that its location was in cells which lacked anionic sites on their luminal surface (e.g., alveolar type I cells). The second, dose independent phase ($30 \text{ min} \leq t \leq 180$ min) was also fastest for F-P(HEA-AA), and consistent with passive diffusion through the alveolar epithelia. In all cases, the smaller molecules of the administered molecular weight distributions were absorbed preferentially and it remained possible that the apparent 30 minute "duration" for the transporter mechanism(s) in the isolated organ, was due to consumption of available energy sources, while active transport may continue in intact animals.

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REFERENCES

1. J. S. Patton. Mechanisms of macromolecule absorption by the lungs. *Adv. Drug Del. Rev.* **19**:3–36 (1996).
2. P. R. Byron, Z. Sun, H. Katayama, and F. Rypacek. Solute absorption from the airways of the isolated rat lung. IV. Mechanisms of absorption of fluorophore-labeled poly-α,β-[N(2-hydroxyethyl)-D,L-aspartamide]. *Pharm. Res.* **11**:221–225 (1994).
3. L. Weiss. The cell periphery. *Int. Rev. Cytol.* **26**:63–105 (1969).
4. D. Simionescu and M. Simionescu. Differential distribution of the cell surface charge on the alveolar-capillary unit. *Microvasc. Res.* **25**:85–100 (1983).
5. C. A. Vaccaro and J. S. Brody. Structural features of alveolar wall basement membrane in the adult rat lung. *J. Cell Biol.* **91**:427–437 (1981).
6. R. W. Niven, F. Rypacek, and P. R. Byron. Solute absorption from the airways of the isolated rat lung. III. Absorption of several peptidase-resistant synthetic polypeptides: Poly-2-(hydroxyethyl)-aspartamides. *Pharm. Res.* **7**:990–994 (1990).
7. P. R. Byron and R. W. Niven. A novel dosing method for drug administration to the airways of the isolated perfused rat lung. *J. Pharm. Sci.* **77**:693–695 (1988).
8. P. R. Byron, N. S. R. Roberts, and A. R. Clark. An isolated, perfused rat lung preparation for the study of aerosolized drug deposition and absorption. *J. Pharm. Sci.* **75**:168–171 (1986).
9. R. W. Niven and P. R. Byron. Solute absorption from the airways of the isolated rat lung. II Effect of surfactants on absorption of fluorescein. *Pharm. Res.* **7**:8–13 (1990).
10. P. R. Byron, H. Katayama, Z. Sun, and F. Rypacek. Airway retention and pulmonary absorption of poly-α,β-[N(2-hydroxyethyl)-D,L-aspartamide]. *J. Biol. Comp. Pol.* **6**:25–35 (1991).
11. P. R. Byron, H. Katayama, Z. Sun, and F. Rypacek. Opportunities for protein delivery by aerosol. *Pol. Prep.* **31**:167–168 (1990).
12. W. W. Yau, J. J. Kirkland, and D. D. Bly. *Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography*, John Wiley & Sons., New York, 1979.
13. G. W. Basset, C. Crone, and G. Saumon. Significance of active ion transport in transalveolar water absorption: a study on isolated rat lung. *J. Physiol.* **384**: 311–324 (1987).
14. E. Ranucci, G. Spagnoli, P. Ferruti, D. Sgouras, and R. Duncan. Poly(amidoamines) with potential as drug carriers: degradation and cellular toxicity. *J. Biomater. Sci. Polym. Ed.* **2**:303–315 (1991).
15. A. Takada, S. Lodhi, N. D. Weiner, and J. Schacht. Lysine and polylysine: correlation of their effects on polyphosphoinisotides in vitro with ototoxic action in vivo. *J. Pharm. Sci.* **71**:1410–1411 (1982).
16. M. W. Seiler, H. G. Rennke, M. A. Venkatachalam, and R. S. Cotran. Pathogenesis of polycation-induced alterations ("fusion") of glomerular epithelium. *Lab. Invest.* **36**:48–61 (1977).
17. A. E. Taylor and K. A. Gaar. Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes. *Am. J. Physiol.* **218**:1133–1140 (1970).
18. T. H. Gardiner and L. S. Schanker. Absorption of disodium cromoglycate from the rat lung: Evidence of carrier transport. *Xenobiotica.* **4**:725–731 (1974).
19. S. J. Enna and L. S. Schanker. Phenol red absorption from the rat lung: Evidence of carrier transport. *Life Sci.* **12**:231–239 (1973).
20. K-J. Kim and E. D. Crandall. Role of endocytic pathways in alveolar epithelial transport of macromolecules. In R. N. Dalby, P. R. Byron and S. J. Farr (eds.) *Respiratory Drug Delivery V.* Interpharm Press, Buffalo Grove, IL, 1996, pp. 153–160.