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# Intrapulmonary Deposition of Aerosolized Evans Blue Dye and Liposomes in an Experimental Porcine Model of Early Ards

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**ABSTRACT**

In early ARDS ( Adult Respiratory Distress Syndrome ) and other inflammatory pulmonary disorders the lung might benefit from a high local deposition of an active drug , in order to optimize the local concentration without systemic side effects. In this methodological study we used pigs under controlled ventilation. The study was carried out in two steps. In the first part Evans blue dye in NaCl was delivered in aerosolized form . In the second part a dry powder containing FITC (fluorescein-isothiocyanate)-labelled-liposomes in NaCl was delivered in the same way. We evaluated whether there was an even, central , peripheral and/or alveolar deposition, whether the procedure was reproducible , and whether there was an interaction with alveolar macrophages. Sixteen animals under chlormethiazole anaesthesia and intermittent positive pressure ventilation (IPPV) were included in the study. Four animals were sacrificed after nebulization and baseline measurements. Five animals served as controls and received saline i.v. Six animals received endotoxin i.v. (18 $\mu g \cdot kg^{-1} \cdot h^{-1}$). One animal underwent broncho-alveolar lavage 15 min and 2 h after liposome administration. At the end of each experiment the lungs were inflated with air, excised and dried in a microwave oven. The left lung of each animal was sliced in a reproducible manner and lung-pieces from different regions were analyzed. The Evans blue dye or the phospholipid fraction of the lungs (containing liposomes),was extracted and analyzed spectrofluorometrically.

This study shows that it is possible,under reproducible conditions,to administer aerosolized Evans blue dye and liposomes and to achieve a deposition in the terminal airways and/or alveolar spaces. The broncho-alveolar lavage demonstrated an interaction of liposomes with alveolar macrophages. The results imply that liposomes carrying active drugs and administered by inhalation may be used for local pulmonary treatment in early ARDS and other related inflammatory pulmonary diseases.

## INTRODUCTION

During the past decade pharmacological therapy , used either as prophylaxis or as treatment, in patients with ARDS (Adult Respiratory Distress Syndrome) and other related inflammatory pulmonary disorders has been of major interest(4,27).The drugs used have been administered primarily by the intravenous route (6,35,39), but a few reports have discussed the possibilty of administering pharmacological substances directly into the lung (32,40).

The advantage of using a local treatment is a high bioavailability i.e. a favourable effect of a proportionately small dose of active drug without the disadvantages of systemic side effects on other organs (5).To a large extent pharmacological substances administered locally into the lung enter the systemic circulation and the substances exhibit pharmacokinetic characteristics very similar to those seen after i.v. administration.

Theoretically, there are several methods which could increase the local bioavailability in the lung. One feasible method is to administer pharmacological substances which are incorporated in liposomes. The carrier potential of liposomes is related to their ability to accommodate water-soluble and lipid-soluble substances in the aqueous and lipid phases, respectively. Certain macromolecules can insert their hydrophobic regions into the lipid bilayers with the hydrophilic portions extending into the water phase (23).

In recent years liposomes have been used to carry antibiotic (2) and antineoplastic drugs (22) to the lung. In these cases the liposomes have been delivered to the lung either by i.v. injection (2,26,36), tracheal instillation (22,29) or in aerozolised form (33). Used in this way the liposomes may also function as slow release preparations in the lung (1,3,14,19).

There are many examples of pulmonary disorders such as ARDS, IPF and various allergic alveolitis in which the lung might benefit from a high local deposition of an active drug, such as a corticosteroid, in order to optimize the local concentration (37).

In these disorders the purpose is to administer a local pharmacological activity to the alveolar spaces , alveolar macrophages and to other cells releasing inflammatory mediators; i.e. cells which are likely to be important in the pathogenesis of these disorders (7).

In order to perform a basic methodological evaluation of the distribution and deposition of aerosolized liposomes in early ARDS, we have chosen an experimental model in the pig (17,28).

This methodological study was carried out in two steps.

In the first part nebulized Evans blue dye was delivered via ventilator to the porcine lung . In the second part FITC (fluorescein-isothiocyanate)-

labelled-liposomes were delivered in the same way.

The aims of this study were to :

1/ characterize the experimental set-up regarding the selected respiratory pattern and the aerosol delivery system.

2/ investigate whether the deposition of Evans blue and liposomes are reproducible and whether there are regional differences in the deposition.

3/ investigate whether it is possible to obtain a deposition in the most peripheral parts of the lung i.e. the alveolar spaces where the major part of the target cells relevant to the pathogenesis of ARDS are located.

4/ investigate whether the pulmonary dysfunction induced by endotoxemia influences the intrapulmonary deposition.

5/ investigate whether liposomes interacts with alveolar macrophages.

## MATERIAL AND METHODS

Preparation and handling of the liposomes

Ten mg of dipalmitoyl-phosphatidyl-choline (DPPC) and 1mg of dipalmitoyl-phosphatidyl-ethanolamine-N-fluorescein-isothiocyanate (DPPE-FITC,Sigma) were mixed in chloroform and dried by evaporation. Then 1 ml of lactose solution (20mg/ml) was added to the lipids and liposomes were formed by sonication using a probe sonicator at 60 °C. The liposomal suspension was fresh frozen by dripping it into liquid nitrogen and subsequently freeze dried. No major changes of the shape and size in distribution were observed when nebulized liposomes obtained from freeze-dried powder were compared with freshly made liposomes.

Aerosol generation

One ml of Evans blue (10 mg /ml,Merck ,Darmstadt ) or a dry powder containing FITC-labelled-liposomes at a concentration of 15.5 mg ( 10 mg lactose , 5 mg DPPC , 0.5 mg DPPC-FITC , AB Draco , Sweden ) were dispersed in 2 ml 0.9 % saline . A fresh liposome preparation was made and actively shaken at 37 °C , 30 min before start of the aerosol generation .

The aerosol was generated from a microaerosol nebulizer (MA2,Viasol,Malmö,Sweden)(9) at a flow rate of 4 $l \cdot min^{-1}$ into the Servo-Ventilator circuit (Fig.1). The temperature of the liposome suspension was kept at 30°C during aerosol generation using a water bath. The nebulizer was placed at a side arm close to the tracheal tube and the aerosol was generated continuously during the whole respiratory cycle.

Thus, both aerosol from the lung and from the nebulizer were collected during expiration on a filter just before the expired air entered the Servo-Ventilator. No increase in the respiratory peak pressure due to the nebulizer flow rate was noticed during nebulization.

Particle size distribution of Evans Blue , FITC-labelled- or placebo(10 mg lactose,5 mg DPPC)-liposomes (primary droplets) , generated either with or without the tubings , was measured by a Malvern Master Sizer ( mass median diameter , MMD ) and dry particles by an Aerodynamic Particle Sizer (mass median aerodynamic diameter , MMAD ).

Animals

Seventeen pigs, of both sexes, of the Swedish native breed, weighing 20-28 kg and aged 12-14 weeks, were used for the study . Only animals with an arterial oxygen tension($PaO_2$)$\geq$ 10 kPa on air and a mean pulmonary arterial pressure(MPAP)$\leq$ 2.7 kPa after catheterization and a 2 h stabilization period, i.e. at baseline , were included in the study.

Anesthesia and catheterization techniques.

All animals were premedicated with pentobarbital 15 mg $\cdot$ $kg^{-1}$ and atropine 0.5 mg intraperitoneally. Anesthesia was induced with ketamine hydrochloride 500 mg i.v. in addition to 0.5 mg atropine i.v. , and steady state anaesthesia was maintained with a continuous i.v. infusion of chlormethiazole 64 mg $\cdot$ $kg^{-1}$ $\cdot$ $h^{-1}$ and pancuronium bromide 0.26 mg $\cdot$ $kg^{-1}$ $\cdot$ $h^{-1}$.

A 0.9 % saline-4 % glucose solution , including the chlormethizole and pancuronium bromide , was administered at a rate of 8 ml $\cdot$ $kg^{-1}$ $\cdot$ $h^{-1}$ throughout the study period. After tracheostomy a 9 mm endotracheal tube was used. A moisture-exchanger (Humidvent R,Gibeck,Sweden) was connected to the endotracheal tube except during the nebulizations. The animals were ventilated mechanically by means of a Servo-Ventilator 900 C (Siemens-Elema) with 70% $N_2O$ in $O_2$ during the preparation and with air during the experiment. To counteract atelectases, hyperinflation with a double tidal volume was performed automatically by the ventilator once every 100 inspiration, except during measurements and nebulizations.

The respiratory rate (RR) was 20 breaths $\cdot$ $min^{-1}$ and the ventilatory minute volume was 20-30 ml $\cdot$ $kg^{-1}$ $\cdot$ $min^{-1}$ in order to obtain a $PaCO_2$ around 4.5 kPa. The inspiratory : postinspiratory pause : expiratory relations were 25% : 10% : 65% . During nebulization the respiratory pattern was altered to 50% : 30% : 20% and the RR was set at 12 $\cdot$ $min^{-1}$.

Arterial, central venous catheters and a 7-Fr Swan-Ganz pulmonary artery catheter were inserted via the right common carotid artery and the right and left external jugular veins , respectively , according to Fig.1. Body temperature was maintained at 38$\pm$1°C by means of a heating lamp.

Parameters relating to lung mechanics,pressures, cardiac output and blood gases were determined as previously described ( 17 ).

Experimental protocol

After completion of the catheterization procedure , a stabilization period of 2 h was allowed. After randomization the animals were subsequently treated according to Tables 1 and 2. Physiological measurements were performed at baseline ("time = 0"), 0.5 , 1.0 , 2.0 , 3.0 and 4.0 h.

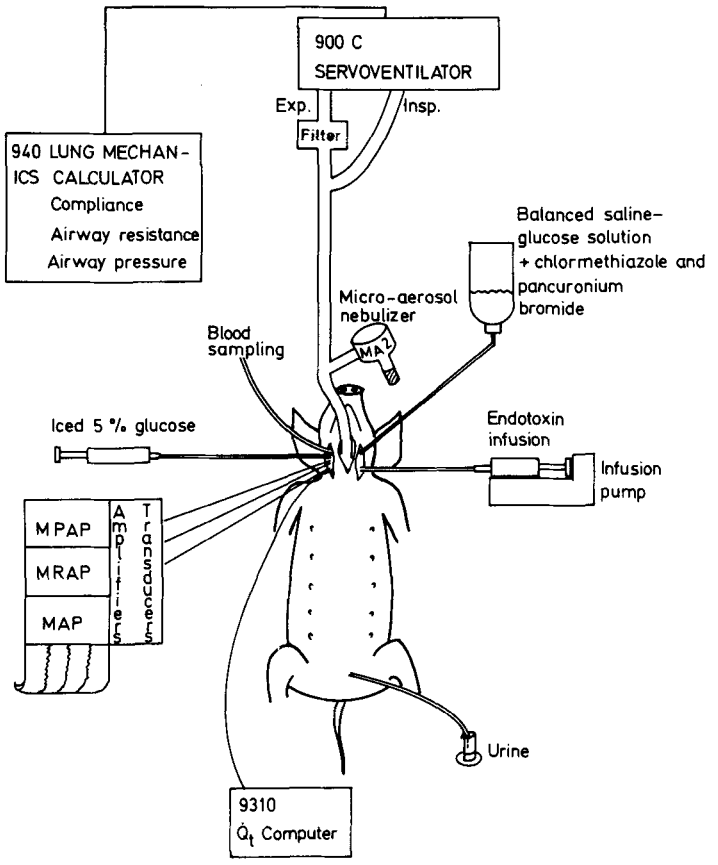


FIGURE 1. The experimental set-up.

Animals receiving Evans blue dye (Table 1):

TABLE 1

Treatment protocol : Evans blue dye

| Pig Number | Time for Nebulization | Sacrified | Infusion |
|---|---|---|---|
| 1 | After baseline measur. time=0 | After neb. | ------ |
| 2 | After baseline measur. time =0 | After neb. | ------ |
| 3 | After baseline measur. time =0 | + 4h | Saline 0-4h |
| 4 | After baseline measur. time =0 | + 4h | Endotoxin 0-4h |
| 5 | +1.5h | After neb. | Saline 0-2h |
| 6 | +1.5h | After neb. | Endotoxin 0-2h |
| 7 | +1.5h | + 4h | Saline 0-4h |
| 8 | +1.5h | + 4h | Endotoxin 0-4h |

Eight animals constituted one group and 4 of these animals were nebulized immediatly after baseline measurements (pigs 1-4). Two of them were sacrified directly after nebulization (pigs 1 and 2) and the other 2 received either an i.v. infusion of 0.9 % saline at a rate of 10 ml $\cdot$ $h^{-1}$ (pig 3) or E.coli endotoxin (0111:B4 prepared as described by Boivin,Difco Labs,Detroit,MI.) in saline over 4 h (pig 4). Four animals were nebulized 1.5 h after start of the saline (pigs 5 and 6) or endotoxin infusion (pigs 7 and 8 ) and sacrified either directly after nebulization (pigs 5 and 7) or 4 h after start of the infusion of saline (pig 6 ) or endotoxin (pig 8 ),respectively. The endotoxin was administered i.v. at a rate of 18 µg $\cdot$ $kg^{-1} \cdot h^{-1}$ { 10 ml $\cdot$ $h^{-1}$ } ( cf. 28 ).

Animals receiving liposomes (Table 2 ):

TABLE 2
Treatment protocol : Liposomes

| Pig Number | Time for Nebulization | Sacrified | Infusion |
|---|---|---|---|
| 9 | After baseline measur. time=0 | After neb. | ------ |
| 10 | After baseline measur. time =0 | After neb. | ------ |
| 11 | After baseline measur. time =0 | + 4h | Saline 0-4h |
| 12 | After baseline measur. time =0 | + 4h | Endotoxin 0-4h |
| 13 | Dead during catheterization | | |
| 14 | +1.5h | After neb. | Endotoxin 0-2h |
| 15 | +1.5h | + 4h | Saline 0-4h |
| 16 | +1.5h | + 4h | Endotoxin 0-4h |
| 17 | After baseline measur. time =0 | After BAL | Saline 0-2h BAL at 15 min and 2h |

Eight animals constituted the other group and received FITC-labelled-liposomes dissolved in saline at a concentration of 7.25 mg / ml (5 mg lactose , 2.5 mg DPPC , 0.25 mg DPPC-FITC ). In this group 4 animals were nebulized immediatly after baseline measurements (pigs 9-12). Two of them were sacrified after nebulization (pigs 9 and 10 ) and the other 2 received either an i.v. infusion of 0.9 % saline at a rate of 10 ml $\cdot h^{-1}$ over 4 h (pig 11) or E.coli endotoxin in saline over 4 h (pig 12). Pig 13 died during catheterization. Three animals were nebulized 1.5 h after start of the saline (pig 15) or endotoxin infusion (Pigs 14 and 16). They were sacrified either directly after nebulization (pig 14) or 4 h after start of saline (pig 15 ) or endotoxin (pig 16 ) infusion , respectively. Pig 17 was infused with saline and underwent bronchoalveolar lavage (BAL) 15 min and 2 h after nebulization. The endotoxin was administered i.v. at a rate of 18 µg $\cdot kg^{-1} \cdot h^{-1}$ { 10 ml $\cdot h^{-1}$ } (cf. 28).

At the end of the experiment the animals in both groups were sacrificed by a controlled exsanguination through the arterial catheter. During exsanguination
Ringer's acetate and 100% $O_2$ were administered in order to obtain maximal depletion of blood before cessation of circulation.

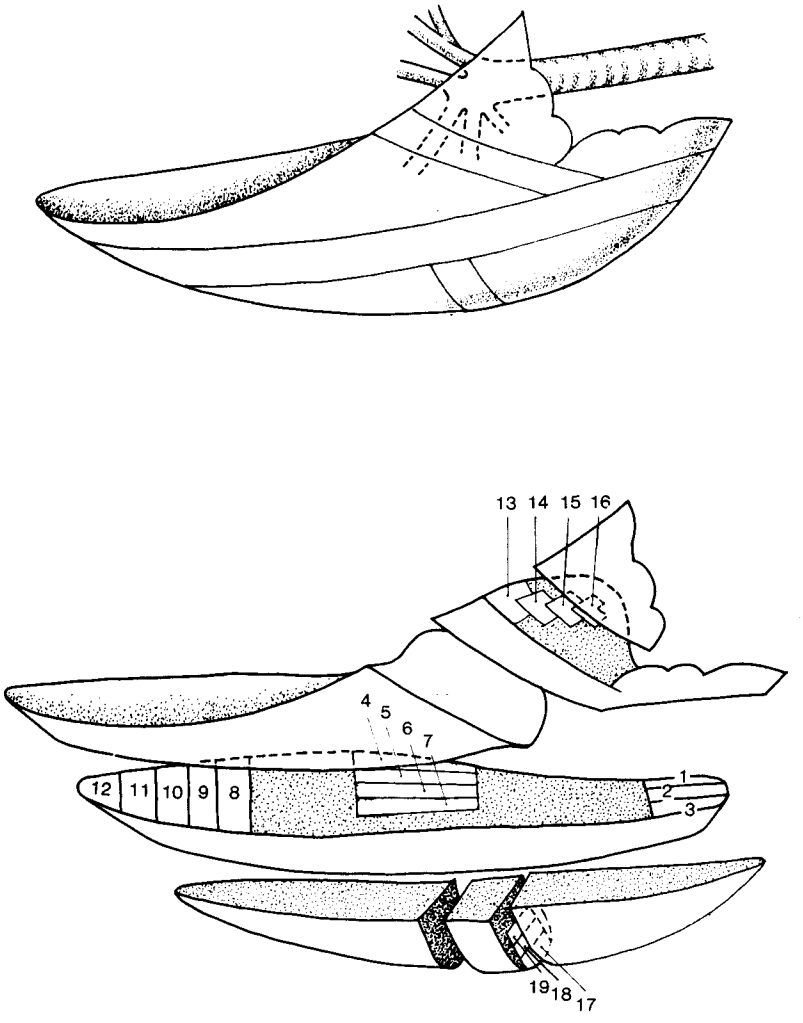


FIGURE 2. Schematic presentation of the left lung , presenting five different regions where representative lung-pieces were cut out.

Preparation of the lungs

At the end of the exsanguination procedure the lungs were inflated with air, carefully excised, suspended , expanded to 30 cm $H_2O$ pressure and desiccated in a microwave owen , essentially according to Valberg et al. (38). The lungs were regarded water-free after 6-7 h without exceeding a temperature of 40 °C.The dried and rigid left lung was carefully sliced in a reproducible manner, parallel to the main bronchus and the lobar bronchi (Fig.2.). As a result , three well defined 1-cm thick slices were obtained from which 19 different lung-pieces were selected. The lung was divided into five regions ( 1-3, apical-peripheral ; 4-7, intermediate ; 8-12, basal-peripheral ; 13-16, central ; 17-19 , intermediate-peripheral).The lung-pieces were excised from the respective slice and their weights adjusted to approximately 100 mg.

Extraction and quantification of Evans blue dye from the lung

Each lungpiece was weighed , shaken in acetone (Kebo lab AB , Sweden ) and $Na_2SO_4$ ( Kebo Lab , Sweden ) (7:3) and extracted for at least 15 h (13). The absorbance of the supernatant was measured on a Hitachi U-3200 at a wave-length of 630 nm .The absorbance per mg dry lung weight of the lung tissue was used as a measure of the distribution of the tracer aerosol in the lung .

Extraction and quantification of liposome deposition

The phospholipid content of the lung was extracted by the Folck procedure (16). Each lung-piece was homogenized together with 8 ml of chloroform/methanol (2:1) in a Potter-Elvehjem homogenizer. The material was put into a polypropylene tube and 2 ml of 0.9 % saline was added. The tube was shaken in a waterbath at 37°C for 2 h and thereafter centrifuged at 4°C,1200 g, for 30 min. The lower phase, which contained all the phospholipid in the sample, was weighed and put in a new polypropylene tube. This sample was allowed to evaporate to dryness for 24 hours. Two ml of 50 mM TRIS-HCl, 5mM $CaCl_2$, pH 7.3 , were added and then the sample was carefully sonicated. Another 3 ml of the same buffer , containing 10 U of phospholipase C (From B.cereus type XIII,Sigma Chemical Company,St.Louis,MISS.), were subsequently added and degradation of phospholipids were allowed during shaking in a waterbath at 37 °C for 24 h.

Thereafter,10 ml of diethylether was added and the sample was shaken for 5 min and centrifuged at 4°C, 3000g for 10 min. The supernatant containing the lipids was aspirated with a pipette. This extraction procedure was repeated twice.From the remaining water/buffer phase, which contained the liberated fluorescein-labelled serine-phosphate, 4 ml aliquots were taken for analysis. The samples were scanned in a spectrofluorometer (Aminco,SPF-500)using 495 nm excitation and 515 nm emission wavelengths. The fluorescence intensity for each sample was related to a reference sample of sodium-

fluorescein.

Broncho-alveolar lavage (BAL)

Under X-ray guidance a thin polyethylene catheter (O.D.=4 mm) was placed in wedge position in the peripheral part of the lung , either through the right (sample 1) or the left (sample 2) main bronchus. Each lavage was done with 2 X 10 ml of 0.9 % saline at 37°C. 2 X 5 ml were recovered. The recovered portions were stored in an icebath, rinsed , diluted in phosphate buffered saline (PBS), and finally centrifuged. The pellets were resuspended in 5 ml of RPMI 16-40 medium and divided into two portions of 2.5 ml each. One portion was placed on a plastic Petri disk and incubated at 37 °C for 30 min to allow for plastic adhesion of the macrophages.The supernatant was decanted and the adherent cells rinsed three times with saline before the Petri disk was studied in an epiilluminated fluorescence microscope. The other portion was studied without purification of plastic adherent cells.

Calculations and statistics

Relations between variables were assesed by linear regression analysis.
M ± SEM are given in the results. P-values < 0.05 were considered significant.

## RESULTS

Particle size distribution

There was no difference in droplet size (MMD) between FITC-labelled- and placebo-liposomes and the MMD measured without tubings was 3.11 µm and 3.13 µm respectively, and with tubings 2.99 µm and 3.17 µm respectively.The MMAD of dry liposomes and Evans Blue particles measured by the APS without tubings was 1.27 µm and 1.20 µm ,respectively, and with the tubings 1.35 µm and 1.21µm, respectively.The geometric standard deviation (GSD) was 1.8 µm . These results indicate that there was no difference in particle size between liposomes and Evans Blue aerosol and the tubings did not change the particle size in the two aerosols.

Spectrofluorometric analysis of the lung tissue samples containing Evans Blue dye .

There was a uniform deposition of Evans blue in the investigated samples. If the mean color dye value (µg) for all samples in an animal was set at 100% , and the deviation from this value was calculated for each piece and taken as an indication of the deposition in that piece , no systematic deviation from the average could be detected for any particular lung area or in the distribution patterns in pigs killed after nebulization at baseline , 1.5 and 4 h , respectively (Fig.3).

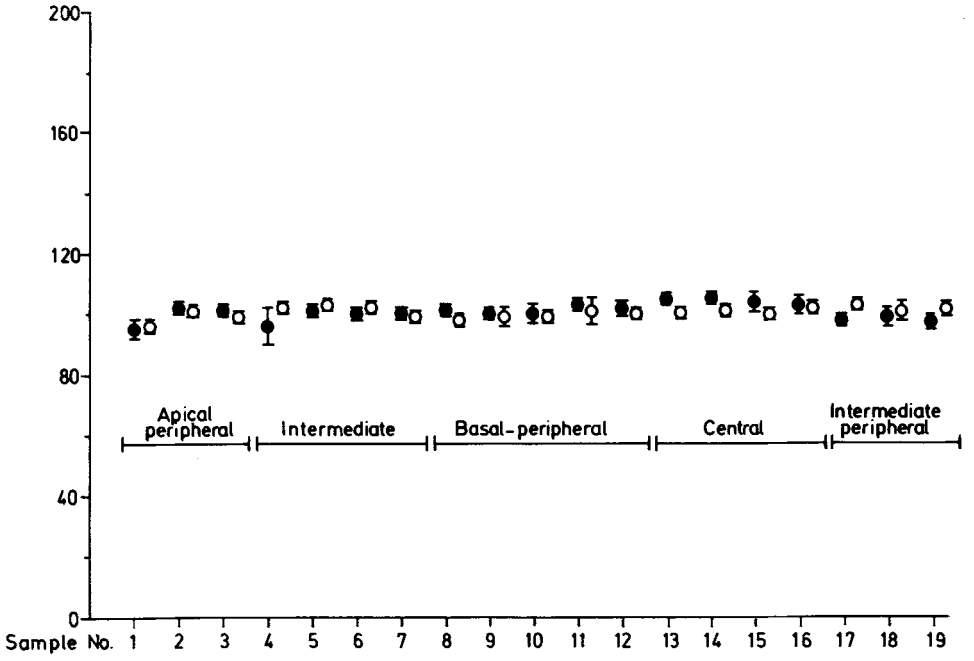


FIGURE 3. Regional distribution of Evans blue dye in the lung regions 1-19 (see Fig.2). The average colour value (µg) of all 19 regions of the lung was set at 100%. Mean±SEM of the relative colour value of each region is shown. o = controls (n=5) ; • = endotoxin animals (n=3)

Further, endotoxin treatment did not seem to have any influence on the intrapulmonary distribution of the fluorescence ( Fig.3.).

As can be seen in Fig.4 , upper panel , the average deposition in the examined lungs varied considerably (min 28.4 µg and max 60.2 µg ).

When related to body weight , however , there was a significant inverse relation between the extent of dye deposition and body weight, thus indicating that the nebulization procedure resulted in a predictable deposition which was reproducible between animals. This was also the case when the average colour value (µg) was related to dry lung weight (Fig.4 ,middle panel ), and is to be expected due to the strict relationship found between dry lung weight and body weight (Fig.4, lower panel ).

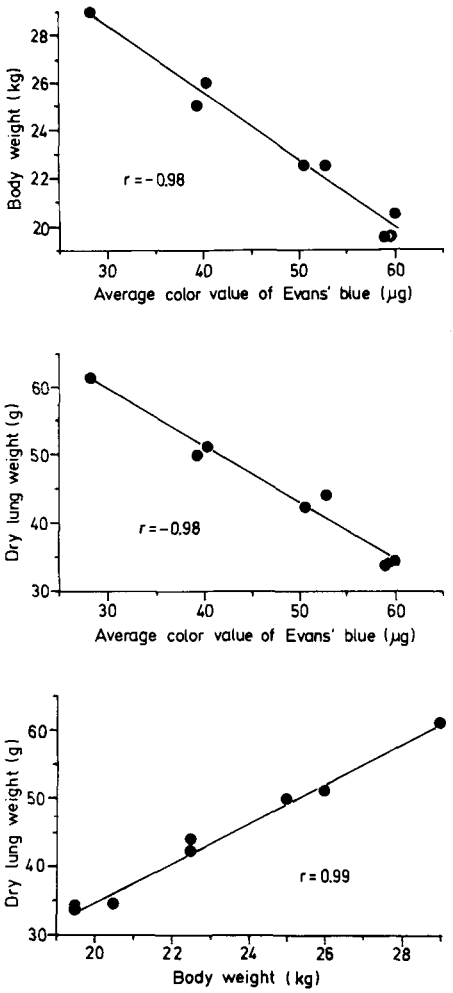


FIGURE 4. Linear correlation between the average measured colour value of Evans blue dye (µg)and the animal weight ( upper panel ); between the average measured colour value and the dry lung weight (middle panel); and between the the animal weight and the dry lung weight (lower panel).

No detectable effects were noted ten minutes after cessation of the nebulization on arterial and mixed venous blood gases , cardiac output , pulmonary haemodynamics and respiratory mechanics.

Spectrofluorometric analysis of the lung tissue samples containing liposomes. There was a fairly uniform but not consistent deposition of flourescein in

the investigated samples. If the mean fluorescence for all samples in an animal was set at 100% , and the deviation from this value was calculated for each piece
and taken as an indication of the deposition in that piece , there was a deviation from the average value in the different lung areas. The same degree in deviation was seen regarding the distribution patterns in pigs sacrificed after nebulization at baseline , 1.5 and 4 h , respectively (Fig.5). Further, endotoxin infusion did seem to have a minor influence on the intrapulmonary distribution of the fluorescence (Fig.5).

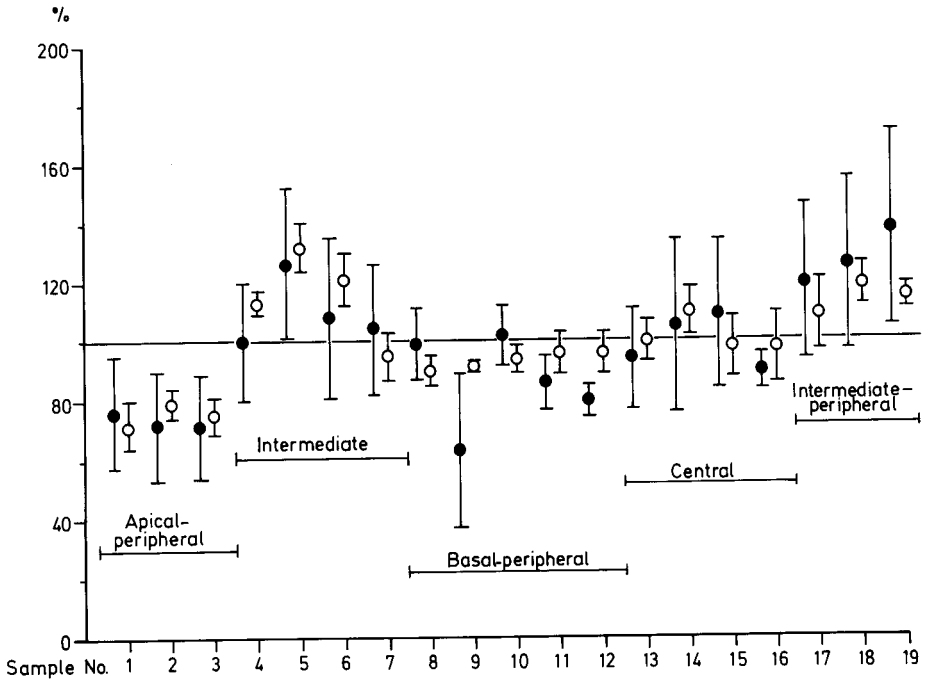


FIGURE 5. Regional distribution of fluorescence in the lung region 1-19 (see Fig.3).The average fluorescence of all 19 regions of the lung was set at 100%. Mean±SEM of the relative fluorescence values of each region is shown. o = controls (n=4) ; • = endotoxin animals (n=3)

As can be seen in Fig.6, upper panel , the average fluorescence deposition in the examined lungs varied considerably (min 17 U and max 35 U).
When related to body weight , however , there was a significant inverse relation between the extent of fluorescence deposition and body weight, thus indicating that the nebulization procedure resulted in a predictable

deposition of liposomes which was reproducible between animals. This was also the case when
the average fluorescence was related to dry lung weight ( Fig.6 , middle panel), and is to be expected due to the strict relationship found between dry lung weight and body weight ( Fig.6, lower panel ). No detectable effects were noted ten minutes after cessation of the nebulization on arterial and mixed venous blood gases , cardiac output , pulmonary haemodynamics and respiratory mechanics.

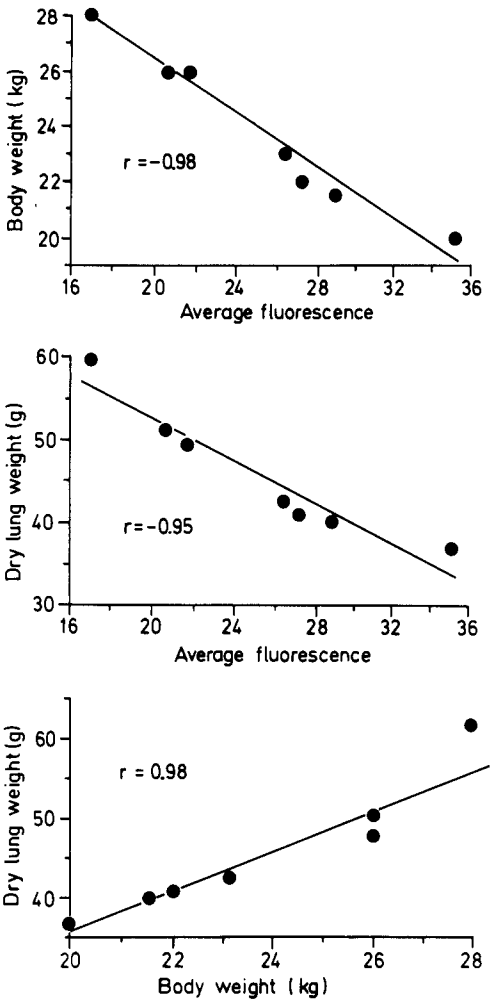


FIGURE 6. Linear correlation between the average measured fluorescence and the animal weight(upper panel); between the average measured fluorescence and the dry lung weight(middle panel); and between the the animal weight and the dry lung weight(lower panel).

Broncho-alveolar lavage

The number of cells in the 4 BAL fluids were approximately 10 x $10^6$ · $ml^{-1}$. A large number of fluorescent particles, with a size of approx. 1 µm , were seen in the fluid of samples taken 15 min after nebulization.In the samples taken at 2 h there was seen a very low extracellular fluorescence. Investigation of plastic adherent cells revealed that approximately 1% of the alveolar macrophages contained fluoroscein 15 min after nebulization (Fig.7) and this increased to 15-20% 2 h after nebulization (Fig.8).

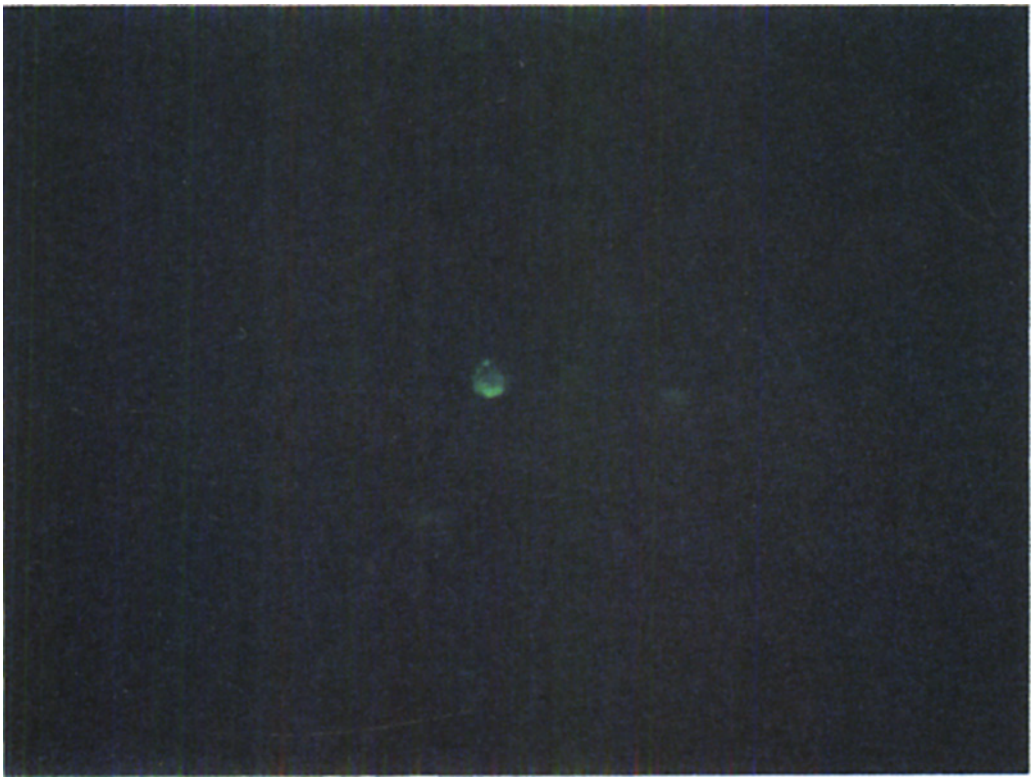

FIGURE 7. Color photo showing fluorescent macrophages 15 min after nebulization .

## DISCUSSION

Inhaled particles are retained at the point where they touch a lung surface.Particles move away from gas streamlines and reach lung surfaces because of several mechanisms:1/sedimentation due to their density,2/ impaction due to their inertia and 3/ diffusion due to their small size. The degree of particle deposition in a given region depends on such factors

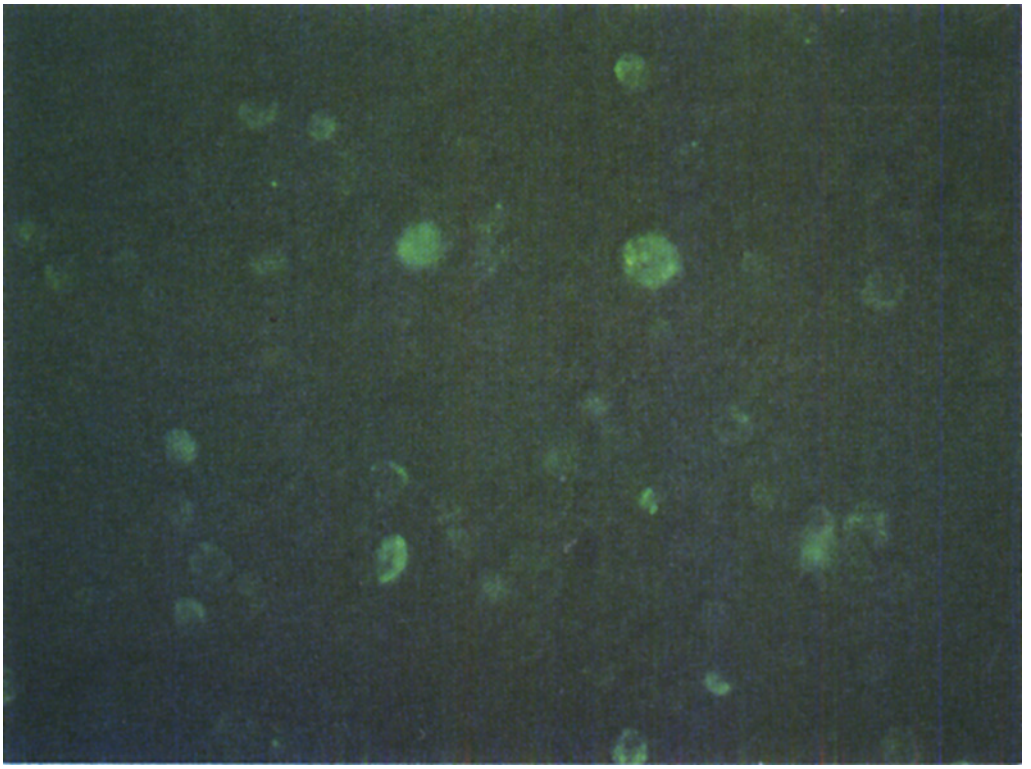

FIGURE 8.Color photo showing fluorescent macrophages 2 h after nebulization.

as the number of particles brought to that region, distribution of ventilation,local airflow velocities and residence times, the angles of airway beanching, and the proximity of lung surfaces. The number of particles deposited may be influenced by the fact that these factors vary from region to region in the lung. The fraction of inhaled particles deposited at a given site describes
local collection efficiency. As airflow pattern and gas residence time can be influenced by altering the ventilatory mode, it is possible that both the fraction of aerosol deposited and the site of deposition can vary ( 10,38 ). Experiments have established that the total deposition within the lung depends on particle size, tidal volume, breathing frequency, ventilatory mode and lung volume ( 8,10,11,12,14,18,21,24,25,31,34 ).Other extensive studies have outlined the factors determining the alveolar clearance of aerosols ( 20,30 ).
Considering the above outlined factors we chose tidal volume , breathing frequncy and ventilatory mode in order to reach an optimal deposition in the alveolar and/or peripheral parts of the lung. Of utmost importance was the

necessity to find a nebulizer that generated a dense cloud of small particles at low flow rates.

Using the above described technique we have found it possible to administer Evans blue dye and FITC-labelled-liposomes in an aerosolized form in a way that leads to a reproducible deposition within the lungs. The Evans blue deposition was evenly distributed within the whole lung whereas the deposition of FITC-labelled-liposomes varied considerably. The data indicates a tendency towards increased deposition of FITC-labelled- liposomes in the "intermediate" and "intermediate-peripheral" regions of the left lung. Since we found no differencies , neither in the particle size distribution of Evans blue or FITC-labelled liposome aerosol, nor the deposition patterns in pigs sacrified after nebulization at baseline , 1.5 and 4 h , respectively , the difference might be explained by methodological errors in the extraction and qantification method of the liposomes.

Another aim of this study was to investigate whether the pulmonary dysfunction related to endotoxin infusion influenced the intrapulmonary distribution. Based on analysis of Evans blue dye and FITC-labelled-liposomes in the different lung-pieces , we did not find any systematic difference in the deposition pattern between the control and endotoxin groups. However, regarding liposome deposition , the spread within each site was larger in the group of animals subjected to endotoxin infusion.

Analysis of the broncho-alveolar lavage following administration of liposomes showed that the liposomes interacted with alveolar macrophages. Since the catheter used was thin and the peripheral wedge position was verified through x-ray , this reflects that the major part of the interactions took place in the alveolar spaces and/or terminal airways.

The results suggest that administration of aerosolized liposomes permits a local deposition in the peripheral airways and alveolar spaces of the lung parenchyma and appears to target alveolar macrophages.

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