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POWDER INHALATION AEROSOL STUDIES II. IN VITRO RAT LUNG MODEL AND ITS COMPARISONS WITH THE AIR SAMPLER

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SUMMARY

An in vitro rat lung model was developed for the evaluation of inhalation aerosols. Several parameters such as the inspired volume, time of inspiration and the amount of drug per inspiration were evaluated. The results of these studies were in agreement with the clinical experiences. The model was compared to the Anderson Air Sampler for the evaluation of drug deposition from powder blends of drug–lactose combinations. The drug deposition pattern in the rat trachea and Stage 3 of the air sampler was similar. Comparisons of the drug deposition in the lungs and Stages 4 through 7 of the air sampler suggested that the rat lung model is a useful tool in evaluating inhalation aerosols. The inhalation parameters evaluated by this model and compared to the clinical results suggest that the model provides good simulation of the respiratory tract.

INTRODUCTION

Knowledge of aerosol particles is important for the optimization of inhalation aerosols in terms of the deposition behavior of fine particles within the human respiratory tract. An extensive literature has been published on the relationship between particle size and the site and extent of deposition in the respiratory tract (Gorman and Hall, 1973). The actual particle size measurements may be based on direct means through the measurement of the particles or their images or by indirect means. The indirect methods are based on laws governing the trajectories of particles, which are a function of size. A wide variety of techniques embodying cyclone disposition, impactor sampling, inertia separation and electrostatic and thermal precipitation, have permitted the development of several instruments. In a number of these methods it is possible only to effect a separation of a sample into a series of size fractions. Additional means are employed to determine particle size of each fraction. In other methods a size analysis can be obtained directly by theoretical treatment or prior calibration of the instrument.

Some investigators have built models of the respiratory tract and determined where the drug was deposited in the model. A simulated lung apparatus composed of 6 compartments based on certain parameters of the human respiratory tract was developed (Karig et al., 1973). To simulate the upper part of the human respiratory tract and the alveolar

parts of the lung, a wet lined pipe and a Seitz type filter were used (Kirk, 1972). Such methods give only relative results and it is difficult to say whether simulation provides a realistic picture.

Impaction devices provide a separation of the material according to the aerodynamic dimensions (diameter X density$^{1/2}$) of the particles. Aerosol particles have been determined by the cascade impactor (Sciara, 1973; Polli et al., 1969; Davis, 1978). A liquid impinger, a modification of the Cascade Impactor, was used in determining the particle size of dry powder aerosols (Bell et al., 1971). An Anderson Air Sampler, a type of Cascade Impactor, was used for determining particle size of inhalation aerosols, produced by pressurized packages with metered valves (Nilsson et al., 1977). For dry powder inhalation aerosols, an Anderson Air Sampler was used to demonstrate the importance of the selection of the proper drug entity in designing inhalation aerosol dosage forms Chowhan and Amaro, 1977). However, the disadvantage of requiring a large sample of the aerosol for accurate determination on lower stages is limited by the assay method and has been discussed earlier (Kirk, 1972; Porush et al., 1960).

In the present studies, an in vitro model utilizing rat lung was developed. Inhalation parameters such as the mode of inhalation, lung volume and total amount inhaled were evaluated and were in agreement with the clinical experiences (Riley et al., 1976; Pavia et al., 1977; Hartley et al., 1977). This model was compared to the Anderson Air Sampler for drug deposition on a series of stages. The results suggest that the rat lung model provides a good simulation of the human respiratory tract.

MATERIALS AND METHODS

Materials

The drug, 7-methylsulfinyl-2-xanthone carboxylic acid (cold and tritiated) was obtained from The Institute of Organic Chemistry (Syntex Research, Palo Alto, Calif.) with a purity of 98.4 and 99.0%, as determined by HPLC and TLC respectively. Potassium hydroxide and hydrochloric acid were analytical reagent grade. Lactose hydrous was USP grade. Polyethylene glycol 400 (Jefferson Chemical Co., Houston, Texas) and monophase-40 (Packard Instrument Co., Downers, Ill.) were also used.

Preparation of inhalation doses

The drug for inhalation was prepared by dissolving tritiated and cold compounds in 0.1 M potassium hydroxide solution at pH 8.0. The solution was quickly acidified by adding a slightly excess amount of 0.1 M hydrochloric acid. The quick acidification is a critical step in controlling the particle size distribution. The coprecipitate was collected by means of a low-speed centrifugation followed by redispersion of the precipitate in 0.01 M hydrochloric acid. The procedure was repeated three times to ensure complete removal of the salt resulting from the acid–base neutralization process. The acidic suspension was cooled in acetone–dry ice mixture for 20 min before lyophilization (Vertis Co., Gardiner, N.Y.). The lyophilization was complete when the temperature and pressure reached minus 80°C and 100 μm Hg, respectively. The freeze drying process prevented aggregation of particles during solvent evaporation and gave particle size distribution similar to micronization. The lyophilized powder was blended with lactose which was

sieved to remove particles smaller than 75 μm in size. The drug lactose blend was filled into #2 hard gelatin capsules.

Rat lung inhalation apparatus

A schematic diagram of the rat lung inhalation apparatus is shown in Fig. 1. It consists of an inhalation device which is connected to an artificial mouth, throat and thorax. The artificial thorax consists of a 500-ml suction flask with a centrally perforated size 7 rubber stopper. The trachea of the rat lung is tied to the terminal part of the throat and the rat lung is suspended in the artificial thorax which is connected to a Bennert-type manometer with a three-way glass stopcock. The manometer is connected to a 1/10 HP vacuum pump which is adjustable to a desirable vacuum. The mouth and the throat were fabricated from pyrex glass with three planes merging half way to the air passage to simulate the pharynx (Fig. 2). The lower portion of the throat was detachable to facilitate coating with a thin film of polyethylene glycol 400 and to prevent blockage of the small opening by excess coating material. The two portions of the throat were joined with a plastic tape. The inhalation device was connected through a Tygon tubing with a one-way pin valve to prevent the incident backflow of an emitted aerosol cloud during the deflation phase of the lung.

Young male Sprague–Dawley-derived rats were used. The animals were sacrificed by a

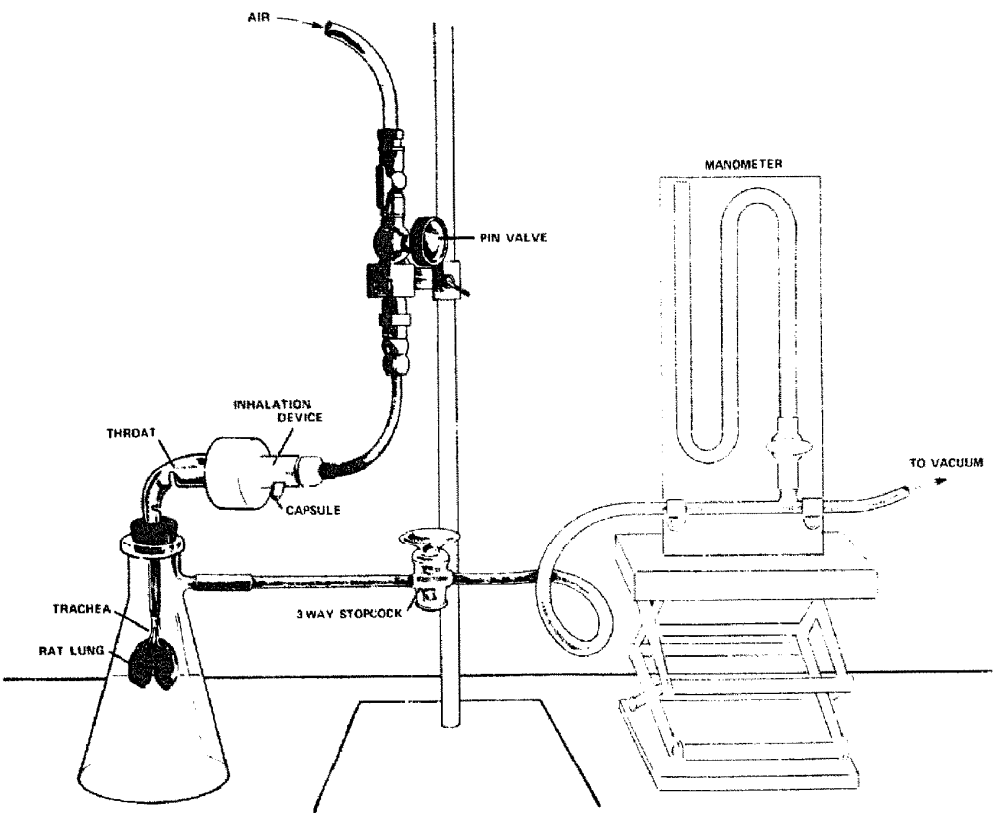

Fig. 1. Schematic diagram of the rat lung inhalation apparatus.

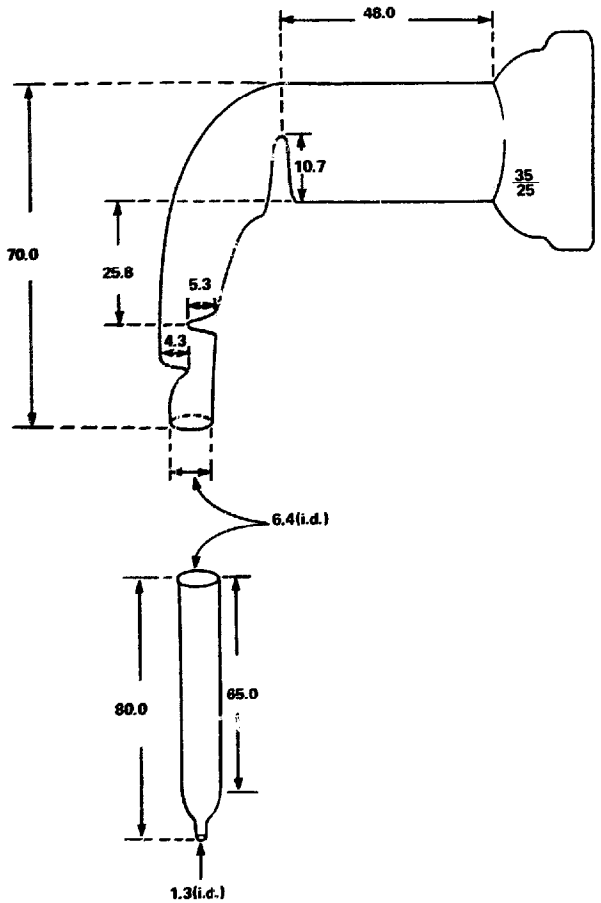

Fig. 2. Schematic diagram of the mouth and throat. Dimensions are given in millimeters.

spinal-disconnecting technique and the lung, including the whole trachea, was isolated and trimmed to remove the surrounding tissues. After rinsing with 0.9% sodium chloride solution, the lung was tied to the terminal opening of the throat by means of a nylon thread.

Inhalation experiment using the rat lung

Six freshly isolated lungs were used for each set of experiments. The vacuum pump was started and the air flow regulated by adjusting the one-way pin valve using a flowmeter for calibration (Roger Gilmont Instruments, Great Neck, N.Y.). After an inspection of tight fitting of all the units with the three-way stopcock set at 'close' position, 50 s were allowed for equilibration before recording the pressure drop from the manometer. The three-way stopcock was rapidly released so that the lung was inflated within 1 s resulting in simultaneous inhalation of the powder aerosol generated from the inhalation device due to a sudden pressure drop. At the end of the inhalation time, the stopcock was returned to its 'close' position and the vacuum pump turned off. Finally, the lung was deflated by a slow operation of the three-way stopcock.

Inhalation experiment with air sampler

The procedure for determining particle size distribution by the air sampler was the same and the schematic representation of the air sampler apparatus showing different impaction stages, throat and inhalation device was described earlier (Chowhan and Amaro, 1977). Briefly, the air flow was controlled with the Gilmont Flowmeter. The cap of the hard gelatin capsule containing the powder blend was removed and the body was inserted into the inhalation device. The inhalation device was then attached to the throat adaptor. The whole system was inspected for a tight fit. The pump was operated for 3 s then the capsule body was removed and set aside, along with the top for rinsing.

Analytical procedures

For the lung model, the capsule, the inhaler, the mouth and the throat were detached and rinsed with 0.1% potassium hydroxide solution into separate petri dish bottoms. The contents of each petri dish were transferred to volumetric flasks of appropriate sizes and diluted to volume with 0.1% potassium hydroxide solution. The lung was divided into three parts by cutting the two branches just below the bifurcated point of the trachea. These parts were placed on a 7 cm Whatman #1 filter paper and dried in a vacuum oven at 60°C. The dried trachea and the lung with the filter paper were pelletized and combusted in a sample oxidizer (Model 306, Packard Instrument). The radioactivity was measured by liquid scintillation counting in 15 ml of monophase-40 (Packard Instrument) with a scintillation counter (Unilux II, Nuclear Chicago, Chicago, Ill.).

Blanks prepared by combusting the filter paper were also obtained between each lung and trachea to minimize the radioactivity carry-over from the preceding sample. Quench factor was calculated by spiking the lung and the filter paper with a standard solution containing a certain amount of radioactivity. Combustion efficiency was obtained by spiking the filter paper and comparing with the blank. The overall correction factors for a single trachea and a lung were 0.953 and 0.925, respectively. These figures were checked for every six trachea and lung samples throughout the combustion. The radioactivity contained in each alkaline washout from the capsule, the inhalation device, the mouth and the throat were counted with the scintillation counter in addition to the absorbance measurement at 342 μm with Unicam SP-1800 Spectrophotometer (Philips Electronic Instruments, Santa Clara, Calif.). Data obtained from two methods were in good agreement.

The assay for inhaled doses with the air sampler was essentially the same as described in the previous report (Chowhan and Amaro, 1977), except that alkaline washout from Stages 4, 5, 6 and 7 were combined because of the relatively small doses used in the present study. The data obtained from radioactivity measurement as well as the spectrophotometric absorbance measurements, were in good agreement.

RESULTS AND DISCUSSION

The results of the effect of the mode of inhalation on regional deposition of the drug in the rat lung model are given in Table 1. At constant air flow rate, an increase in the inhalation time resulting in an increase in the inspired volume did not increase the deposition of the drug in the lungs. These changes, however, did increase the drug deposition in the trachea. Thus an increase in the inspired air volume resulted in an overall increase in

TABLE 1

EFFECT OF INHALATION PATTERN ON REGIONAL DEPOSITION ON THE DRUG IN THE RAT LUNG MODEL

Airflow (liter/min)	Time (s)	Apparent volume [b] inspired (ml)	Normalized percent deposition from drug lactose 1 : 1 blend [a]				
			Capsule	Inhalor	Mouth and throat	Trachea	Lung
4.2	5	350	0.78 (±0.29)	12.91 (±2.55)	74.96 (±2.95)	8.64 (±0.43)	2.71 (±0.82)
4.2	3	210	1.21 (±0.22)	15.97 (±2.88)	73.09 (±2.75)	7.58 (±0.77)	2.16 (±0.73)
7.0	3	350	1.22 (±0.25)	11.04 (±5.91)	77.13 (±5.67)	9.16 (±1.10)	1.44 (±0.88)
					t test at $P = 0.05$ level; d.f. = 10		
			(1) versus (2):		$t = 1.140$ insignificant	$t = 2.976$ significant	$t = 1.217$ insignificant
			(2) versus (3):		$t = 1.572$ insignificant	$t = 2.893$ significant	$t = 1.544$ insignificant
			(3) versus (1):		$t = 0.832$ insignificant	$t = 1.075$ insignificant	$t = 2.585$ significant

[a] Total recoveries from (1), (2), and (3) are 100.42, 102.24 and 103.37%, respectively.
[b] Calculated as: volume inspired (ml) = airflow (liter/min) × 1000 ÷ 60 × time (s).

the combined percent drug deposition in the trachea and in the lung. Similar results were obtained when the inhalation time was kept constant and the volume of the inspired air was increased by increasing the flow rate. These results are in agreement with the clinical situation, where an increase in the tidal volume resulted in an increase in the drug penetration (Pavia et al., 1977).

Higher drug deposition in the lungs resulted from lower flow rate, longer inspiration time and constant inspired volume (Table 1). This is in agreement with the regional deposition studies in man (Lippman and Albert, 1969; Goldberg and Lourenco, 1973), which indicate that the main inhalation variable which affects the depth of penetration is the flow rate during inhalation.

Table 2 gives the effect of dose on regional deposition of the drug in the rat lung model. The percent drug deposition in the lungs is not significantly different from 2.5 mg and 5 mg doses. However, a higher percentage of the drug was deposited in the trachea from smaller doses. The average amount of the drug deposited in the trachea and lung of the rat at 2.5 mg dose was 284 μg and at 5 mg dose was 442 μg.

These results are in general agreement with a salbutamol study in man in which the bronchodilator effect was evaluated in 10 asthmatic patients (Hartley et al., 1977). Four hundred μg of the powder aerosol gave approximately 2-fold increase in percent peak expiratory flow rate compared to the 200 μg dose.

The results of the effect of dose on particle size distribution of the drug in the air

TABLE 2

EFFECT OF DOSE ON REGIONAL DEPOSITION IN THE RAT LUNG MODEL [a]

Dose [c] (mg)	Normalized percent deposition from drug lactose 1 : 1 blend [b]				
	Capsule	Inhalor	Mouth and throat	Trachea	Lung
2.5	0.78 (±0.29)	12.91 (±2.55)	74.96 (±2.95)	8.64 (±0.43)	2.71 (±0.82)
5.0	0.32 (±0.08)	17.58 (±0.34)	73.26 (±2.39)	6.68 (±0.55)	2.16 (±0.37)
				t-test at $P = 0.05$ level; d.f. = 10	
				$t = 6.906$ significant	$t = 1.472$ insignificant

[a] Airflow = 4.2 liter/min; time = 5 s; vacuum = 24 ± 0.1 cm Hg.
[b] Total recoveries from 2.5 and 5.0 mg doses are 101.54 and 97.42%, respectively.
[c] The drug lactose blend is assumed to have an absolute homogeneity.

sampler are given in Table 3. The percent drug deposition on Stage 3 and Stages 4 through 7 at 2.5 mg and 5 mg dose was similar. This is in agreement with the rat lung model except that the percentages of drug deposition are higher in the air sampler.

Drug and lactose blends containing zero to five parts of lactose were used to study the drug deposition behavior in the rat lung model and in the air sampler. Fig. 3 gives the normalized percent drug deposition in various parts of the rat lung model and in the air sampler. The deposition of the drug in the two models is similar with Stage 3 in the air

TABLE 3

EFFECT OF DOSE ON PARTICLE SIZE DISTRIBUTION OF THE DRUG IN THE AIR SAMPLER [a]

Dose [c] (mg)	Normalized percent deposition from drug lactose 1 : 1 blend [b]				
	Capsule	Inhalor	Throat → Stage 2	Stage 3	Stages 4 → 7
2.5	2.94 (±0.46)	15.64 (±0.53)	61.69 (±1.55)	11.00 (±0.65)	8.74 (±1.32)
5.0	1.58 (±0.39)	12.63 (±1.71)	66.63 (±2.18)	10.87 (±1.67)	8.33 (±0.78)
				t-test at $P = 0.05$ level; d.f. = 6	
				$t = 0.174$ insignificant	$t = 0.534$ insignificant

[a] Airflow = 29 liter/min; time = 3 s.
[b] Total recoveries from 2.5 and 5.0 mg doses are 101.83 and 97.19%, respectively.
[c] The drug lactose blend is assumed to have an absolute homogeneity.

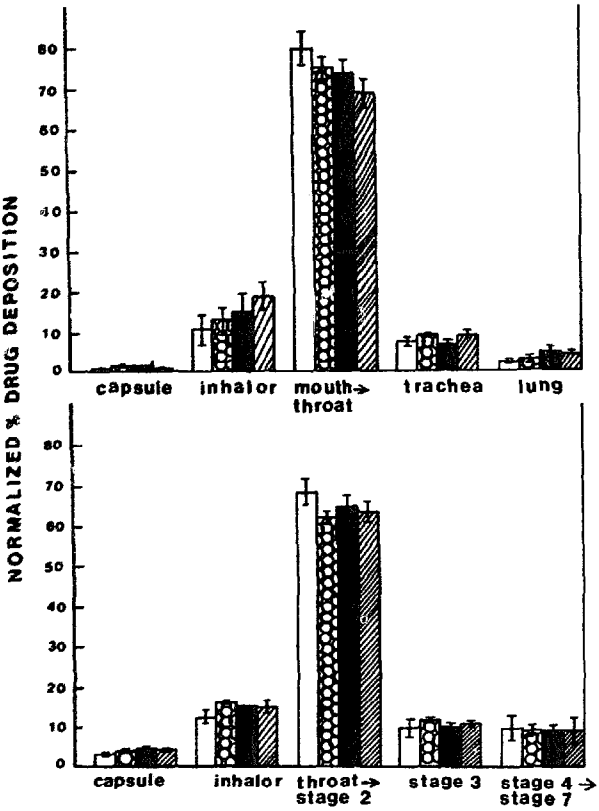

Fig. 3. Normalized percent drug deposition in the air sampler and in the rat lung model. Key: □, 2.5 mg drug; ▨, 2.5 mg drug + 2.5 mg lactose; ■, 2.5 mg drug + 7.5 mg lactose; ▩, 2.5 mg drug + 12.5 mg lactose. Vertical bars represent standard deviations.

sampler, corresponding to the trachea and Stages 4 through 7 corresponding to the lung. On this basis of comparison, the air sampler gives higher percent drug deposition compared to the lung and trachea in the rat model.

Fig. 4 compares the drug deposition in the trachea of the rat lung model and Stage 3 of the air sampler from various drug–lactose blends. The pattern of the drug deposition in the trachea and Stage 3 in both models is similar. Higher amounts of lactose in the formula did not increase the deposition of the drug. Less drug was deposited in the rat trachea compared to Stage 3 of the air sampler for all drug–lactose blends.

The results of drug deposition in the rat lung and Stages 4 through 7 of the air sampler from drug–lactose blends are given in Fig. 5. The percent drug deposition remained unchanged in the air sampler as the proportion of the lactose in the formula increased at 2.5 mg dose. At 10 mg dose level, increase in the amount of lactose resulted in an increase in the deposition on Stages 4 through 7 (Chowhan and Amaro, 1977). This suggests that the air sampler becomes less sensitive to formulation factors, as the dose is decreased.

At 2.5 mg dose level, the rat lung model gave higher percent drug deposition in the lung, as the lactose ratio in the formulation was increased. This suggests that the rat lung model would be a more useful tool in the evaluation of the formulation parameters for small dose aerosols.

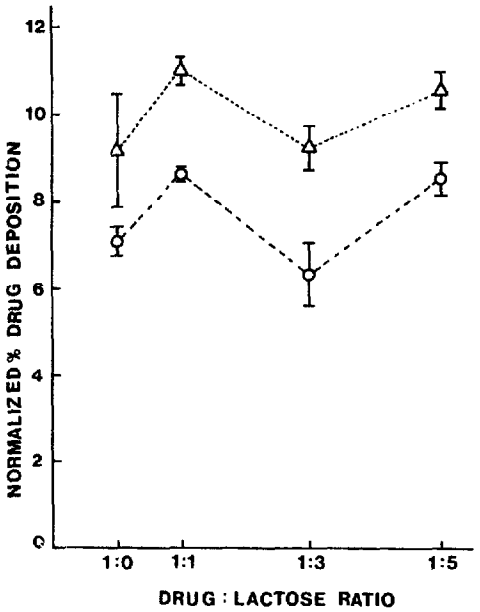

Fig. 4. (Left) Effect of the drug: lactose ratio on the normalized percent drug deposition on Stage 3 of the air sampler and in the trachea of the rat lung mode. Key: △, Stage 3 of the air sampler; ○, trachea of the rat lung model. Vertical bars represent standard errors.

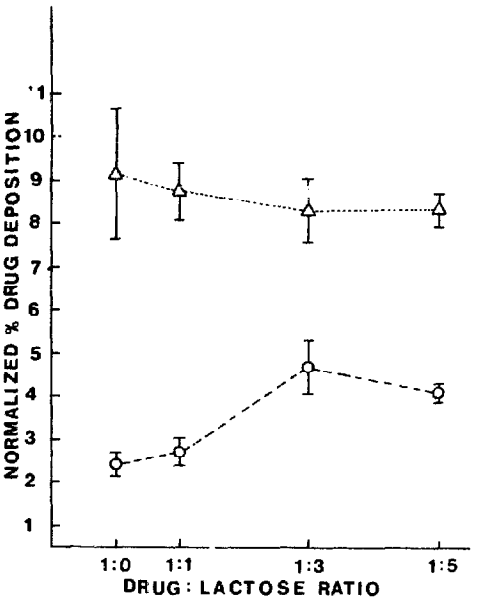

Fig. 5. (Right) Effect of the drug: lactose ratio on the normalized percent drug deposition on Stages 4 through 7 of the air sampler and in the lung of the rat lung model. Key: △, Stages 4 through 7 of the air sampler; ○, lung of the rat lung model. Vertical bars represent standard errors.

It is important to note that the air sampler consistently gave higher percentages of drug distribution on Stages 3 through 7 compared to the deposition in the trachea and the lung in the rat model. Although there are anatomical differences between the lungs of man and rat (Laskin, 1972), there is some justification in the expectation that the rat lung model may more closely parallel what would be expected with bronchial delivery of drug to man than results with the air sampler device. Since there is very little literature regarding in vitro–in vivo correlation with bronchial drugs, this is an area of investigation deserving future experimental effort and clarification.

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