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Tissue Distribution and Metabolism of Drugs. IV.[1] Accumulation and Penetration of Some Antibiotics in Rat Lungs[2]

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The lung accumulation and permeation of some antibiotics were studied using an isolated blood-perfused rat lung preparation with artificial ventilation and the intact lung of anesthetized rats *in vivo*. Leucomycin A_3 and erythromycin, which have been widely used for the treatment of pulmonary infections, were accumulated well in the lung, while tetracycline and chloramphenicol did not show specific accumulation. The specific accumulation of leucomycin A_3 and erythromycin is probably due to the fact that both drugs have an amino group and a strongly lipophilic group. In the case of intratracheal administration in the perfused lung, the transport of these antibiotics from alveoli to the blood was shown to be dependent on their lipid solubility, and the drug distribution in the lung was found to be similar to that from the perfusate in the equilibrium state. However, in the *in vivo* distribution study, marked accumulation of erythromycin after intratracheal administration was observed as compared with intravenous administration, whereas, the distribution of leucomycin A_3 was not greatly affected by the route of administration. On the basis of these experimental findings, drug delivery systems are discussed in relation to the effective distribution of drugs in the lung.

Keywords——tetracycline; chloramphenicol; erythromycin; leucomycin A_3; lung; drug distribution; lung accumulation; lung absorption; lipid solubility

Antibiotic agents are administered by various routes in the treatment of bronchopulmonary infections in man.[4] The effect of administration route on the fate of drugs used in the treatment of such infections is thus of particular interest.[5] Knowledge of the pharmacokinetic parameters that govern drug distribution and metabolism in the lung seems necessary to ensure optimal treatment or to prevent adverse reactions.

In our earlier paper,[1] the accumulation of various drugs in the lung was monitored in isolated perfused rat lungs with artificial ventilation, and it was suggested that a cationic group as well as a lipophilic group in the molecule were required for the specific accumulation of drugs. However, few reports are available on lung uptake and biotransformation of antibiotics used in the treatment of pulmonary infections. The purpose of this investigation was therefore to ascertain whether our hypothesis is applicable to antibiotics and to clarify the relationship of lung uptake to the penetration of some antibiotics in the isolated perfused lung and in the intact lung *in vivo*.

Materials and Methods

Materials——Leucomycin A_3 and erythromycin were kindly supplied by Toyo Jozo Co. Ltd., Tokyo. Tetracycline, ^{3}H-tetracycline and chloramphenicol were obtained from commercial sources. All other materials were of analytical reagent grade.

1) Part III: K. Okumura, H. Yoshida, and R. Hori, *J. Pharm. Dyn.*, **1**, 230 (1978).
2) Part of this work was presented at the 9th Symposium on Drug Metabolism and Action, Kumamoto, November, 1977.
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4) *a*) Y. Hiro and Y. Doi, *J. Antibiotics ser-B*, **22**, 85 (1967); *b*) K. Fukaya, M. Hayakawa, and O. Kitamoto, *Chemotherapy*, **18**, 252 (1970); *c*) K. Nitta, *J. Antibiotics ser-A*, **20**, 181 (1967); *d*) K. Kanamori, *Chemotherapy*, **5**, 83 (1957).
5) *a*) M. Kawai, *Chemotherapy*, **23**, 3141 (1975); *b*) H. Iwamoto, *Nihon University Medical Journal*, **35**, 551 (1976).

Animals——Male Wistar rats weighing 180 to 220 g were used in all experiments.

Media for Lung Perfusion——The perfusion medium (10 ml) was a mixture of fresh rat blood and Krebs-Ringer bicarbonate buffer (1:1), equilibrated with carbogen gas (O_2 95%, CO_2 5%) before perfusion.

Lung Perfusion Procedure for Drug Accumulation and Absorption *in Vitro*——The perfusion method for isolated lung was that described in our previous paper.[1] The isolated lung was perfused at a rate of 8 ml/min and ventilated by negative pressure 60 times/min for 60 min. In the case of lung accumulation studies, drug solutions of various concentrations were added to the perfusate and drug clearance from the perfusate was measured. For the absorption studies, drug solution was administered into the trachea, then drug appearance in the perfusate was monitored.

Procedure for Distribution Experiments *in Vivo*——Intravenous and intratracheal administration were used. The intratracheal drug administration technique was similar to that described by Schanker *et al.*[6] The drug was given to anesthetized rats, then blood samples were collected from the carotid artery at specified times. Sixty minutes after drug administration, rats were exsanguinated and the lung, liver, kidney and spleen were removed for measurement of drug content.

Analytical Methods——A. Leucomycin A_3 and Erythromycin: These macrolide antibiotics in the perfusate or tissue were analyzed by measuring their antibacterial activities by the paper disc method using *Sarcina lutea* ATCC 9341 as the microbial strain.

B. ^{3}H-Tetracycline: The lung perfusate and lung homogenate containing ^{3}H-tetracycline were oxidized using a sample oxidizer (model 306, Packard Instrument Co.), and the product, ^3H_2O, was solubilized in a scintillation cocktail (monophase-40, Packard Instrument Co.). Radioactivity was determined with a Tri-Carb liquid scintillation spectrometer (model 3330, Packard Instrument Co.).

C. Chloramphenicol: Chloramphenicol was determined by the method of Yamazaki.[7]

Determination of Drug Metabolites in the Perfusate and Lung Tissue——A. Leucomycin A_3 and Erythromycin: These antibiotics and their metabolites in the lung homogenate or perfusate were concentrated after deproteinization and chromatographed on Silica Gel F Spotfilm (Tokyo Kasei Co.) in the following solvent systems: chloroform–methanol–ethylacetate–water (v/v, 59:11:8:2) for leucomycin A_3; chloroform–methanol–2% ammonia water (v/v, 50:50:1) for erythromycin. After development, the plate was placed on an agar medium for bioautography.

B. ^{3}H-Tetracycline: The perfusate or lung homogenate was deproteinized with methanol, and the supernatant was concentrated by evaporation, then chromatographed on Silica Gel G using butanol–methanol–14% citric acid aqueous solution (v/v, 4:1:2) as a solvent. After development, the radioactivity of the thin-layer plate was checked with a liquid scintillation spectrometer.

C. Chloramphenicol: The perfusate or lung homogenate was extracted with chloroform, and the organic layer was evaporated down. A small amount of concentrated extract was applied on a Silica Gel G plate and developed in chloroform–methanol–14% ammonia water (v/v, 10:4.5:1). Next, the ultraviolet (UV) (265 nm) absorption was determined with a dual-wavelength thin-layer chromatography scanner (Shimadzu CS-900).

Results and Discussion

Accumulation of Some Antibiotics in the Isolated Perfused Rat Lung

In order to determine the distribution characteristics of antibiotics in the lung, the accumulation of ^{3}H-tetracycline, chloramphenicol, erythromycin and leucomycin A_3 in the perfused lung was studied. Fig. 1 shows the clearance curves of these drugs. When leucomycin A_3 and erythromycin were allowed to recirculate through the isolated lung, large amounts of these antibiotics were accumulated in the lung and disappeared from the circulation, as shown in Fig. 1C—D. Leucomycin A_3 was better accumulated in the perfused lung at lower concentrations, as was reported previously for many basic drugs with high lipid solubilities.[1] However, the accumulation of chloramphenicol was low, and ^{3}H-tetracycline was hardly accumulated (Fig. 1A—B). This might be due to their poor lipid solubilities and lack of basicity. For further investigation of the dose dependency of drug accumulation in the lung, the concentration ratio of these antibiotics in the lung after perfusion for 60 min with respect to the perfusate concentration of unbound drugs was expressed as a function of initial concentration in the perfusate, as shown in Fig. 2. It was clearly demonstrated that leucomycin A_3 was accumulated in the isolated lung in a dose-dependent manner, while

6) S.J. Enna and L.S. Schanker, *Am. J. Physiol.*, **222**, 409 (1972).
7) M. Yamazaki, S. Shigeo, and A. Kamada, *Yakuzaigaku*, **34**, 97 (1974).

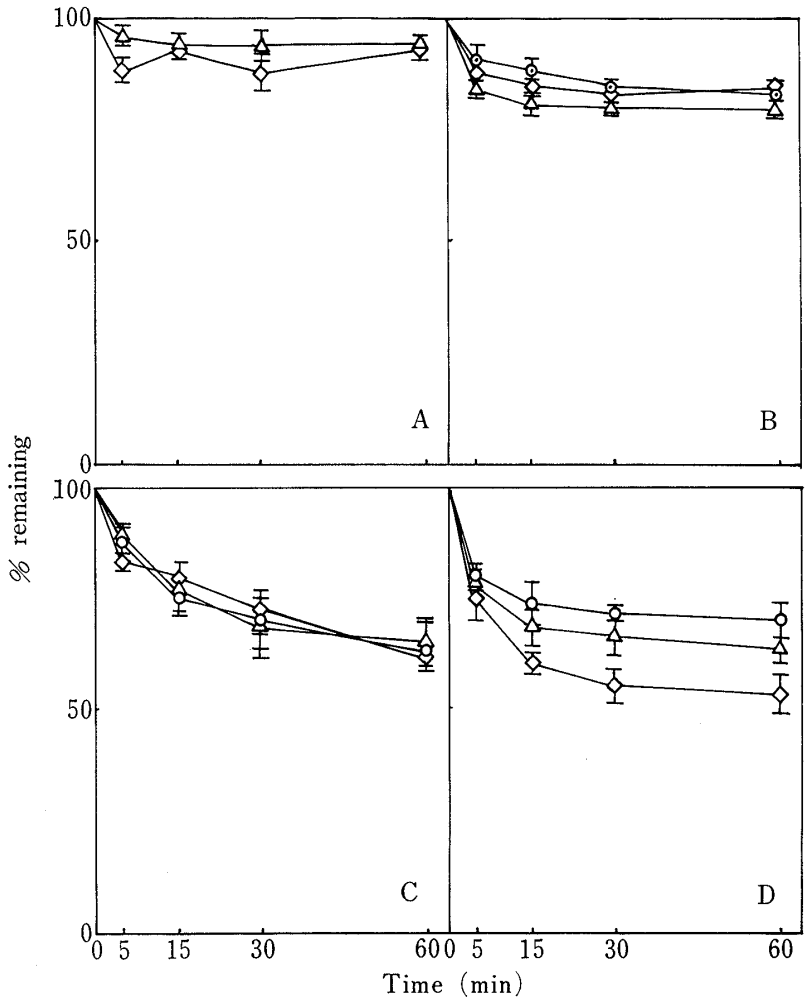

Fig. 1. Time Course of Drug Clearance from the Perfusate

A, tetracycline; B, chloramphenicol; C, erythromycin; D, leucomycin A_3.
Initial concentration of drug in the perfusate: ⊙, 2 mM; ○, 1 mM; △, 0.2 mM; ◇, 0.05 mM.
Each point represent the mean ± S.E.M. of three to six experiments.

the other three antibiotics were accumulated with a constant lung/plasma ratio within this concentration range. To confirm these results, the metabolic fate of these antibiotics was studied. The extract of lung perfusate or lung homogenate was chromatographed on thin-layer plates in a suitable solvent system, and the metabolites were determined by bioautography, spectrophotometry and radioactivity assay. As shown in Fig. 3 and 4, erythromycin, chloramphenicol and tetracycline yielded no detectable metabolite in the lung perfusate and lung homogenate. In the case of leucomycin A_3, two bactericidal metabolites were detected in the lung homogenate, while none was detected in the lung perfusate. However, bactericidal activity in the lung homogenate appeared to depend largely on unchanged leucomycin A_3, because each metabolite spot was very small. The total recoveries were 93, 99, and 100% at concentrations of 0.05, 0.2, and 1 mM, respectively. Thus, it was considered that the drug content shown in Fig. 1 and 2 represent the parent compound, and that the dose-dependency of leucomycin A_3 in lung accumulation cannot be explained by its metabolism. Recent work in our laboratory has suggested that a cationic group and a lipophilic group in the molecule are necessary for specific accumulation of drugs in the lung. The antibiotics used in the present study, except for chloramphenicol, are weak bases with pK_a values ranging from 6.7 to 10.2 and have partition coefficients ($CHCl_3/H_2O$) of 4500 for leucomycin A_3, 150 for erythromycin and 0.06 for tetracycline. When the percent accumulation within 60 minutes in the perfused lung was plotted against the partition

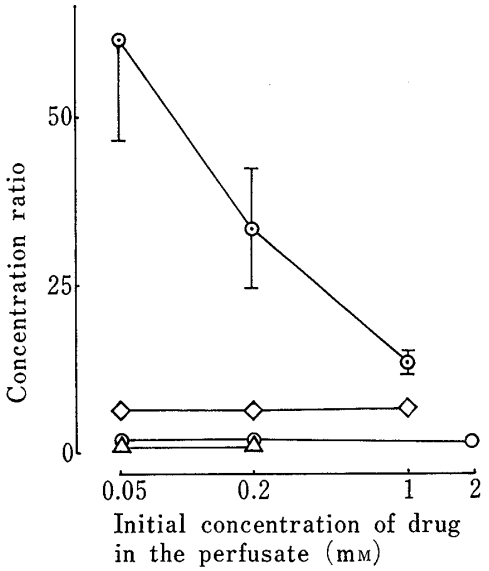

Fig. 2. Concentration Ratios of Drugs in the Lung to Unbound Drug in the Perfusate after Perfusion for 60 min in the Isolated Lung

⊙, leucomycin A_3; ◇, erythromycin; ○, chloramphenicol; △, tetracycline.

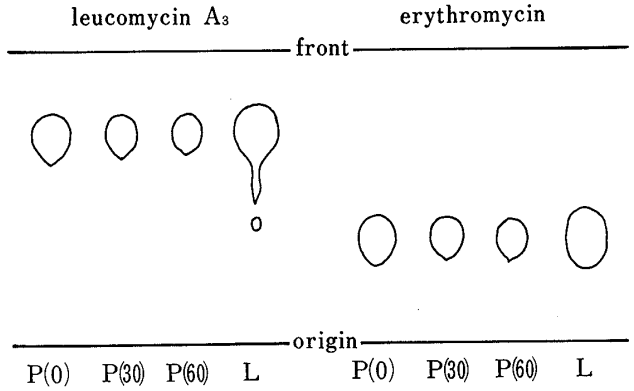

Fig. 3. Bioautograms of Leucomycin A_3 and Erythromycin in the Perfusate and Lung

L, lung; P, perfusate.
Numbers in parentheses refer to the sampling time (min).
The initial concentration of drug in the perfusate was 0.05 mM.

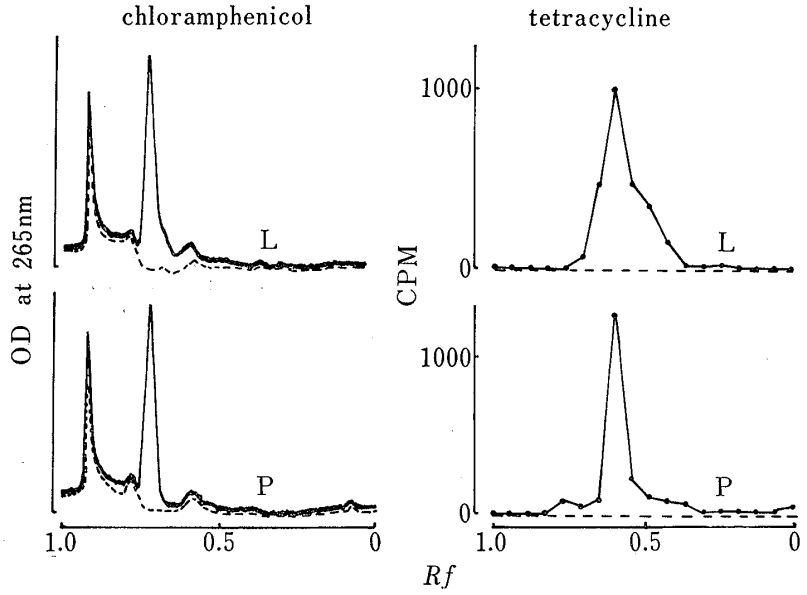

Fig. 4. Thin-Layer Chromatograms of Extracts of the Perfusate and Lung after Perfusion for 60 min with ^{3}H-Tetracycline or Chloramphenicol

L, lung; P, perfusate.
The initial concentration of drug in the perfusate was 0.05 mM.
Broken lines show samples without drug.

coefficient, there was good correlation. These data demonstrate that the most important factors for lung accumulation of these antibiotics are the basicity and lipid solubility of the drugs at physiological pH. In general, macrolide antibiotics such as erythromycin and leucomycin A_3 are widely used in the chemotherapy of pulmonary infections, and are trapped in the lung. Our results might aid in developing an understanding of the drug action of these antibiotics.

The Transfer of Antibiotics from the Alveolus in the Isolated Perfused Lung

In spite of the popularity of oral antibiotics, inhalation therapy or nasal administration of antibiotics is still used in clinics. It is of interest, therefore, to investigate whether drug accumulation is affected by the route of administration. Although the rapid transport and specific accumulation of basic antibiotics from blood vessels to the lung tissue were clearly demonstrated, they have not yet been confirmed for basic drugs after intratracheal administration. In view of this, the accumulation and transport of some antibiotics from the alveoli to lung tissue or lung perfusate were examined in the isolated perfused lung. Fig. 5 shows the time courses of drug appearance in the lung perfusate (solid line) after intratracheal injection of leucomycin A_3, erythromycin, chloramphenicol and tetracycline. Leucomycin A_3

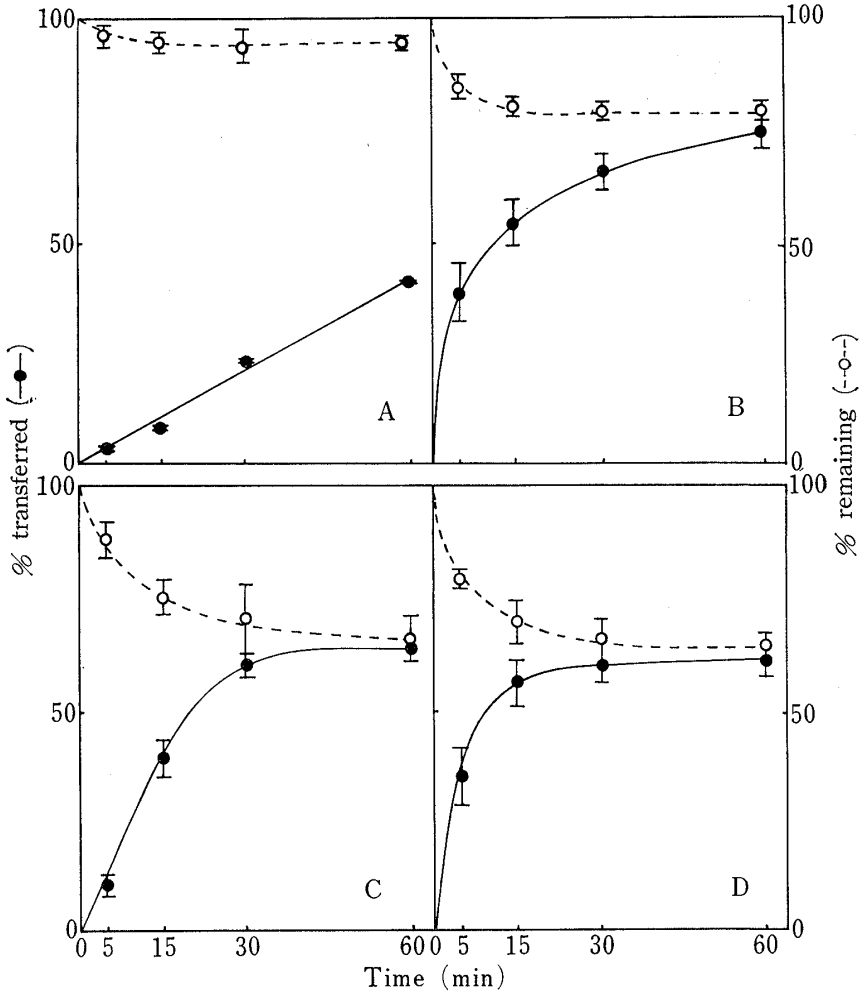

Fig. 5. Time Courses of Penetration of Drugs from the Perfused Lung to the Perfusate after Drug Instillation into the Trachea

A, tetracycline; B, chloramphenicol; C, erythromycin; D, leucomycin A_3.
○, drug was administrated into the perfusate.
●, drug was administrated into the trachea.
Dose, 2 μmol.
Each point represents the mean±S.E.M. of four to six experiments.

appeared rapidly in the lung perfusate and reached equilibrium within 15 min. Erythromycin was transported from alveoli to the lung perfusate at a slower rate than leucomycin A_3, and reached equilibrium within 60 min. On the other hand, the data for chloramphenicol and tetracycline did not indicate a plateau even after perfusion for 60 min, indicating that some unabsorbed drug still remained in the trachea or alveoli at that time. According to Schanker

et al.,[8] the transport of a drug from alveoli to the blood depends largely on its lipid solubilities. Our results also showed a good correlation between the transfer rates of antibiotics and their lipid solubilities. For comparison, the time courses of drug disappearance from lung perfusate to which the same amounts of antibiotics had been added are shown by dotted lines. It appears that the transport barrier (mainly a lipoidal barrier) for absorption of these antibiotics in the lung is probably located in the alveolar epithelium, because the drug accumulation from the blood to lung tissue equilibrated quickly, as described above. The transport characteristics of tetracycline, which is poorly lipid-soluble, seem similar to those reported for the transalveolar transport of large polar solutes such as sucrose, inulin, and dextran, which permeate through the alveolar epithelium *via* water-filled channels.[9] Thus, it seems that the lipoidal barrier and a system of channels are located in the alveolar epithelium. At the end of this experiment, the alveoli were flushed out twice with 2 ml of physiological saline *via* the trachea, after which the drug content in the lung was measured. The concentration ratios of lung to unbound perfusate for leucomycin A_3 and erythromycin after perfusion for 60 min were 27.6 and 7.8, respectively. In the case of drug accumulation from the lung perfusate, the concentration ratios of lung to unbound perfusate were 33.2 for leucomycin A_3 and 6.4 for erythromycin, as shown in Fig. 2. Accordingly, the effect of administration route on the lung accumulation of leucomycin A_3 and erythromycin *in vitro* was not marked in this equilibrium system.

Distribution of Antibiotics after Intratracheal Administration *in Vivo*

In contrast with the tissue distribution of drugs in the isolated perfused lung, the distribution pattern of drugs in the lung after intratracheal administration *in vivo* may be complex, because a state of equilibrium cannot be achieved. Therefore, it seems desirable to determine the distribution patterns of antibiotics after intratracheal administration of

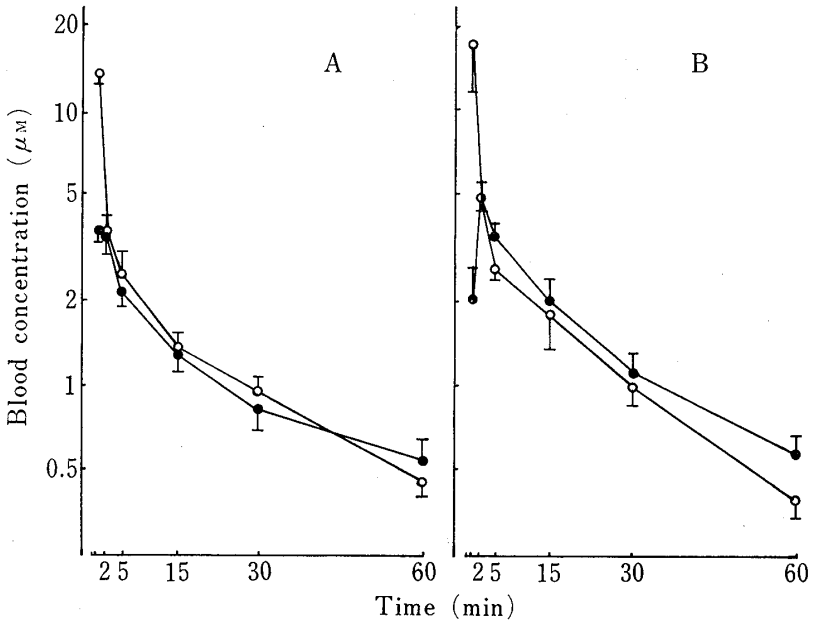

Fig. 6. Blood Concentration Profiles of Antibiotics after Intravenous or Intratracheal Administration

A, leucomycin A_3; B, erythromycin.
Dose, 5 μmol/kg body weight.
Each point represents the mean ± S.E.M. of four to five experiments.
●, drug was injected intratracheally.
○, drug was injected intravenously.

8) S.J. Enna and L.S. Schanker, *Am. J. Physiol.*, **223**, 1227 (1972).
9) J. Theodore, E.d. Robin, R. Gaudio, and J. Acevedo, *Am. J. Physiol.*, **229**, 989 (1975).

Table I. Tissue-to-Plasma Ratios of Drug Concentration 60 min after Drug Administration

	Leucomycin A_3 5 μmol/kg		Erythromycin 5 μmol/kg		Erythromycin 1.25 μmol/kg	
	i.t.	*i.v.*	*i.t.*	*i.v.*	*i.t.*	*i.v.*
Lung	16.9±2.2	10.4±1.1	169.9±39.9	13.1±1.8	161.5±30.0	8.1±2.1
Liver	——	——	5.9± 1.0	6.4±0.8	4.2± 0.8	4.2±0.7
Kidney	3.6±0.3	4.7±0.7	10.2± 1.3	15.0±2.5	8.5± 0.9	11.2±3.0
Spleen	6.2±0.4	6.3±0.5	10.4± 1.4	14.5±2.5	11.7± 2.2	10.1±2.2

Each value represents the mean ± S.E.M. of four to nine experiments.

drugs *in vivo*. Fig. 6 shows the time courses of drug concentration in the plasma after intratracheal of intravenous administration of leucomycin A_3 and erythromycin. No significant difference in the blood concentration profiles was observed between intravenous and intratracheal administration of leucomycin A_3, as shown in Fig. 6-A. Almost the same results were obtained in the case of erythromycin (Fig. 6-B). These results suggest the rapid permeation of these antibiotics across the epithelium of the respiratory tract. It also appeared that leucomycin A_3 was absorbed more quickly than erythromycin, and this rapid absorption of leucomycin A_3 was in accord with the results of the *in vitro* experiments (Fig. 5). The concentration ratios of tissue to plasma (T/P) of these antibiotics 60 min after administration are also shown in Table I, indicating high tissue concentration compared to the plasma concentration. The results indicate that accumulation of leucomycin A_3 is significantly higher in the lung than in the kidney ($p<0.001$) and the spleen ($p<0.01$), while that of erythromycin in the lung is almost identical to the levels in the kidney and spleen after intravenous administration. The higher lipid solubility of leucomycin A_3 as compared to erythromycin may account for its high affinity for the lung. A very high lung-to-plasma concentration ratio of erythromycin was obtained after intratracheal administration as compared to that after intravenous administration. However, the lung-to-plasma concentration ratio of leucomycin A_3 after intratracheal administration was only 50% higher than that after intravenous administration. These results suggest that the lung distribution of some macrolide antibiotics may be affected by the route of administration of pharmaceutical preparations. In order to exclude the possibility that the high concentrations of intratracheally administration drugs in the lungs may depend on the presence of unabsorbed drug, the residual amount of antibiotics in the alveolar space was determined. After a 60-minute distribution experiment, the alveolar side of the lung was flushed out with physiological saline and the drug level in the wash was determined. In spite of careful washing, no detectable antibiotic was found. Thus, the high concentration of erythromycin in the lung after intratracheal administration cannot be explained in terms of unabsorbed erythromycin in the alveolar space. Although some workers have reported the presence of a high concentration of macrolide antibiotics in the lung after intranasal administration[10] or inhalation,[11] there are few quantitative data on lung accumulation after drug administration to the respiratory tract *in vivo*. As a dynamic process may contribute to this process, a pharmacokinetic analysis of the distribution pattern in the lung after intratracheal administration is currently in progress in this laboratory. The data presented here suggest that some antibiotics such as erythromycin would be more effective if they were administered *via* the trachea in chemotherapy for lung infections. Thus, the development of a drug delivery system for the respiratory tract, such as powder or aerosol inhalation, might be useful for antibiotic therapy.

10) O. Kitamoto, K. Fukaya, A. Kitta, A. Kawashima, and H. Honma, *J. Antibiotics ser-B*, **15**, 1 (1962).
11) N. Maekawa, S. Tsukuma, M. Nakanishi, M. Kawai, F. Kuse, M. Yamashita, and M. Kanda, *J. Antibiotics ser-B*, **22**, 140 (1967).