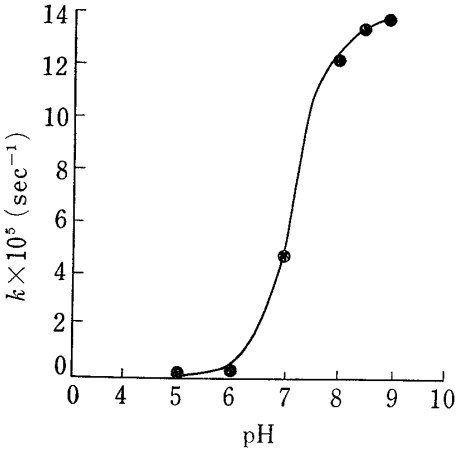

Fig. 3. pH Dependence of the Inactivation of Lipoprotein Lipase from *Pseudomonas fluorescens* by Methylene Blue-catalyzed Photooxidation

Photooxidation was performed at 37° for 2 hr with 0.063% protein and 0.0032% methylene blue.
k: the pseudo-first order rate constant for the inactivation of lipoprotein lipase.

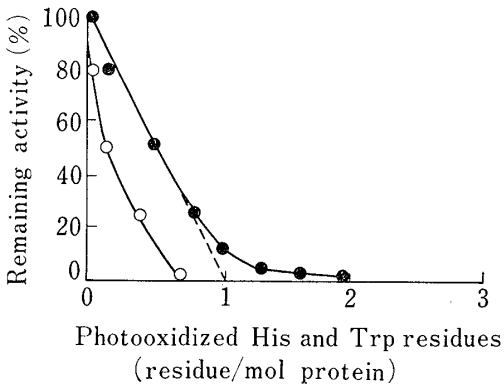

Fig. 4. The Relationship between the Lipoprotein Lipase Activity and the Amount of Photooxidized Histidine and Tryptophan Residues in the Lipoprotein Lipase from *Pseudomonas fluorescens*

For details, see the text.
—●—: His residue, —○—: Trp residue.

in the enzyme activity. In addition, no disapperance of other amino acid residues such as tyrosine was observed, and methionine sulfoxide formation was not detected. Thus, it appears that one histidine residue is specifically involved in the catalytic activity of LPL.

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Enhancement of Pulmonary Absorption of Fluorescein Isothiocyanate-Labelled Dextran (FITC-dextran) by Sodium Glycocholate in Rats

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The effects of a sulfonic acid dye and a surfactant on the pulmonary absorption of fluorescein isothiocyanate-labelled dextran (FITC-dextran), a model macromolecular compound (MW=150000) which is not absorbed after pulmonary administration, were studied. Bromphenol blue, a sulfonic acid dye, did not show an enhancing effect. However, sodium glycocholate (SGC) considerably enhanced the pulmonary absorption of FITC-dextran. Therefore, SGC may be a useful additive to increase the systemic availability of macromolecular compounds administered as aerosol dosage forms.

Keywords——absorption; intrabronchial administration; macromolecular compound; sodium glycocholate; rat

Recently, much attention has been paid to the feasibility of intrabronchial administration of drugs such as heparin,[2)] salbutamol,[3)] disodium cromoglycate,[4)] cyanocobalamin,[5)]

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gentamicin[6] and kanamycin,[7] which are not absorbed or are absorbed very slowly from the gastrointestinal tract. Previous work in this laboratory dealing with the absorption of macromolecular compounds such as enzymes, proteins and carbohydrates from rat lung has indicated that most macromolecular compounds are well absorbed from the pulmonary site, except for the highest molecular weight dextran (MW=150000).[8]

On the other hand, it has been reported by Schanker[9] that intrabronchially administered organic anionic dyes markedly increase the absorption rates of *p*-aminohippuric acid, tetraethylammonium and mannitol. In addition, Hirai[10] suggested that the absorption of insulin from the nasal mucosa is enhanced by the addition of a surfactant, sodium glycocholate.

Therefore, in this study, we investigated the absorption of FITC-dextran (MW=150000) upon coadministration of an organic anionic dye or a surfactant.

Experimental

FITC-dextran (MW=150000) was purchased from Pharmacia Fine Chemicals. Bromphenol blue (BPB) and sodium glycocholate (SGC) were obtained from Wako Chemical Industry, Osaka. The test solution for intrabronchial administration of FITC-dextran was prepared by adding 5 mg of FITC-dextran to 0.9% sodium chloride solution. Other test solutions were prepared by adding BPB (10 mM) or SGC (0.01 mM, 0.1 mM, 1 mM and 10 mM) to the FITC-dextran test solution. Eighteen male Wistar rats weighing 300 g to 400 g were divided into six groups. One group (three rats), designated as Group A, received only FITC-dextran as a control experiment. The other five groups, B, C, D, E and F, received FITC-dextran together with BPB or SGC. Group B received FITC-dextran with 10 mM BPB, group C received FITC-dextran with 0.01 mM SGC, and groups D, E and F received FITC-dextran with 0.1 mM, 1 mM and 10 mM SGC, respectively. To investigate the pulmonary absorption of FITC-dextran, rats were anesthetized by means of an intraperitoneal injection of sodium pentobarbital and 0.1 ml of the test solution was administered intrabronchially (5 mg/300 g), according to the method developed by Schanker.[11] Blood was collected from a cut at the end of the tail at various times up to seven hours after the administration. In another experiment, the same amount of FITC-dextran was injected intravenously into two rats amd blood was collected similarly. Serum FITC-dextran concentration was measured spectrophotometrically, as described in our previous report.[8]

Results and Discussion

When FITC-dextran was administered (5 mg/300 g) intrabronchially to rats (Group A), no fluorescence was detected in the serum up to 24 hours. Therefore, this serum concentration *vs.* time curve is not shown. Recently, Schanker[9] reported that intrabronchially administered sulfonic acidic dye, BPB (5—10 mM), markedly enhanced the pulmonary absorption rates of many compounds, including phenol red (0.1 mM), *p*-aminohippuric acid (0.1 mM), tetraethylammonium (0.1 mM) and mannitol (0.1 mM). However, in this work, when BPB (10 mM) was coadministered intrabronchially with FITC-dextran (0.33 mM) to rats (Group B), no fluorescence was detected in the serum up to 24 hours after the administration. Therefore, it appears that BPB has no effect on the pulmonary absorption of FITC-dextran.

Hirai[10] reported that nasal absorption of insulin from a recently developed aerosol dosage form[12] was considerably enhanced by the coadministration of SGC. Therefore, SGC was added to the test FITC-dextran solution at four different concentrations, 0.01 mM, 0.1 mM, 1 mM and 10 mM. After intrabronchial administration to Groups C, D, E and F, serum was collected for up to seven hours. The results are shown in the figure. When a

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small amount of SGC (0.01 mM) was added to the test solution, fluorescence due to FITC-dextran was detected in the serum after the intrabronchial administration. Thus, the pulmonary absorption of FITC-dextran was enhanced by the addition of SGC. When the amount of SGC added to the test solution was increased to 0.1 mM, the serum FITC-dextran level was also increased and reached a peak earlier than seven hours after intrabronchial administration. In the case of 1 mM SGC, the enhancing effect of SGC on the pulmonary absorption of FITC-dextran was also seen, but when the amount of SGC was increased to 10 mM, the serum FITC-dextran level was decreased.

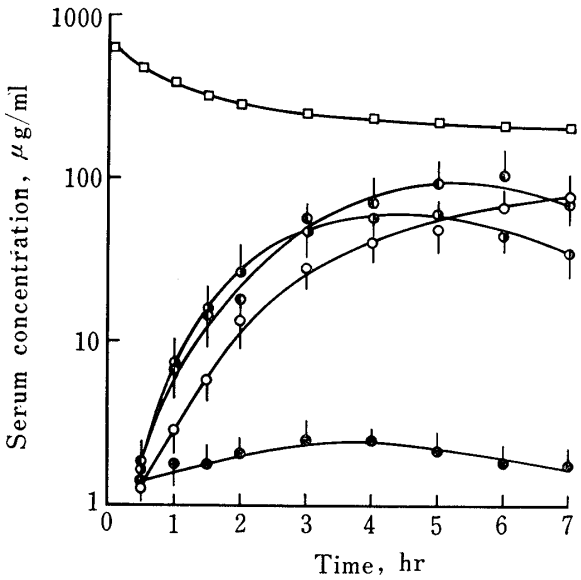

Fig. 1. Serum FITC-dextran Concentration *vs.* Time Curves after Intravenous (□) and Intrabronchial (various circles) Administration to Rats at a Dose of 5 mg/300 g with Various Amounts of Sodium Glycocholate; 0.01 mM (○), 0.1 mM (◑), 1 mM (◐) and 10 mM (●)
Vertical bars represent SD.

The concave curve shows the disappearance rate of FITC-dextran from the blood stream after the intravenous administration of the same amount of FITC-dextran, 5 mg/300 g (each point in the figure shows the mean serum FITC-dextran level of two rats). By comparing this concave curve with the other convex curves, it can be seen that pulmonary absorption of FITC-dextran is considerably enhanced by the coadministration of 0.01 mM, 0.1 mM or 1 mM SGC. As the concentration of SGC was increased from 0.01 mM to 1 mM, the enhancing effect of SGC on the pulmonary absorption of FITC-dextran increased. However, from the standpoint of dosage form design, a sufficient effect on the pulmonary absorption of FITC-dextran is obtained at the lowest dose, 0.01 mM SGC.

Though the effect of SGC on the pulmonary absorption was examined with only one model compound, FITC-dextran (MW=150000), which is not absorbed by itself *via* the pulmonary route, SGC should also be useful for the pulmonary administration of other macromolecular drugs, enzymes, proteins and carbohydrates as aerosol dosage forms. Such bioactive macromolecules often have little or no side effect, but it is difficult to use them as drugs because of their poor absorbability from the administered site. SGC may be useful as an additive to increase the systemic availability of these drugs.