# Possible Mechanism Regulating Barrier Function of Rat Intestinal Mucosa against Permeation of Cefmetazole, a Hydrophilic Drug

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**Abstract:** The nonsurfactant adjuvants diethyl maleate (DEM) and diethyl ethoxymethylenemalonate (DEEMM) were previously shown to enhance the colonic absorption of the hydrophilic drug cefmetazole in rats and to concomitantly decrease the nonprotein sulfhydryl concentration of colonic tissue. To test further an association between nonprotein sulfhydryl concentration and membrane permeability, the effects of several adjuvants, DEM, DEEMM, ethanol and sodium salicylate, were tested in the everted sac preparation of rat colon and jejunum. There was a good correlation between decreased nonprotein sulfhydryl concentration and enhanced cefmetazole absorption in both tissues. Moreover, the addition of cysteamine reversed the effects of each adjuvant on nonprotein sulfhydryls and cefmetazole absorption. These results suggest that tissue levels of nonprotein sulfhydryls regulate at least in part the intestinal membrane permeability.

Lipophobic compounds are poorly absorbed from the intestine, because intact intestinal mucosal membranes do not allow the passive diffusion of poorly lipid soluble compounds. However, damaged epithelial cells of the intestine allow free permeation of many water soluble compounds such as trypan blue (1). Essential water soluble compounds require special transport mechanisms to pass such barriers, whereas lipid soluble compounds may often pass by simple diffusion. It is important to clarify which constituents of the intact intestinal membrane are essential in regulating the permeability of a wide range of solutes including nutrients and drugs.

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Recently it has been reported (1, 2) that sodium salicylate modifies the barrier function of the rat rectal mucosa against the permeation of trypan blue and theophylline in living epithelial cells. Furthermore, diethyl maleate and diethyl ethoxymethylenemalonate were previously shown to enhance rectal absorption of cefmetazole and to decrease nonprotein sulfhydryl levels (3). In the present study, the effects of various adjuvants on intestinal membrane permeability, concentration of nonprotein sulfhydryls and tissue protein content were measured in rats with the use of an *in vitro* everted sac method in order to determine whether a direct relationship exist between nonprotein sulfhydryls and membrane permeability.

## Materials and Methods

### *Materials*

Sodium cefmetazole was supplied by Sankyo Co., Ltd. (Tokyo, Japan). Diethyl maleate (DEM) and cysteamine were purchased from Sigma Co., Ltd. (Mo., USA). Diethyl ethoxymethylenemalonate (DEEMM), ethanol and sodium salicylate were purchased from Nakarai Chemicals Co., Ltd. (Kyoto, Japan). Other reagents used were of analytical grade.

### *Animal and Intestinal Everted Sac Preparation*

Wistar male rats, 200 to 225 g, were fasted for 16 h prior to experiments. Animals were sacrified by decapitation with subsequent infusion of 50 ml saline into the aorta to flush residual blood from the intestine. Then, segments of jejunum and colon were removed and everted in iced Krebs-Ringer's solution saturated with oxygen : carbon dioxide (95 : 5). Cefmetazole transport was determined according to the method described by Riegelman et al. (4). The everted sac containing 1 ml Krebs-Ringer's solution was placed in 10 ml Krebs-Ringer's solution saturated with oxygen : carbon dioxide (95 : 5) and incubated at 37°C. Transport of cefmetazole from the mucosal side to the serosal side was determined by measuring the cefmetazole concentration within the sac after 45 min. Transport of cefmetazole was expressed as the ratio of cefmetazole concentration on the serosal side against the initial cefmetazole concentration (0.01 M) on the mucosal side. After the transport study, each everted sac was weighed (wet weight) and was homogenized in saline. The homogenized sample was used for the assay of reduced nonprotein sulfhydryls (RNS) and protein in the homogenate.

### *Analytical Procedures*

Cefmetazole was determined with a high pressure liquid chromatographic method (5), while RNS were measured by the method of Ellmann (6) with glutathione as a standard. The protein assay employed the cumasin blue method described by Bradford (7).

## Results and Discussion

The permeation of cefmetazole through both rat jejunal and colonic sac from the mucosal to the serosal side was minimal in control experiments (Figs. 1 A and 2 A). However, the presence of adjuvants, such as DEM, DEEMM, ethanol, or salicylate on the mucosal side significantly increased the transport of cefmetazole with a simultaneous reduction in the concentration of reduced nonprotein sulfhydryls (RNS) in both jejunal and colonic tissues. Moreover, when adjuvant was added only on the serosal side, cefmetazole transport from the mucosal side was similarly enhanced, again with a concurrent decrease in the RNS tissue concentration (Figs. 1 A and 2 A). In this study, we examined the relationship between reduced rather than total nonprotein sulfhydryl levels and intestinal permeability against cef-

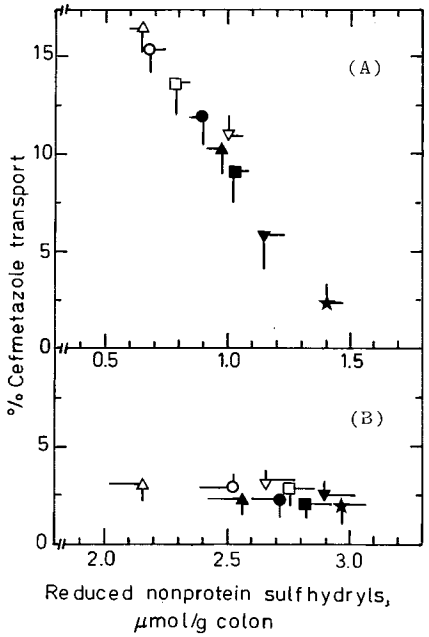


**Fig. 1** Relationship between reduced nonprotein sulfhydryl levels in rat colonic tissue and rat colonic tissue permeability against cefmetazole. A. The effects of diethylmaleate (3 mM, ○ and ●), ethanol (10 %, △ and ▲), sodium salicylate (12.5 mM, ▽ and ▼) and diethyl ethoxymethylenemalonate (3.5 mM, □ and ■) were determined with each adjuvant present on the mucosal side (open symbols) or the serosal side (closed symbols). Permeability was determined as % cefmetazole transported from the mucosal to the serosal side. The symbol ★ represents control values in the absence of adjuvant. B. Effect of cysteamine on the adjuvant action shown in A.
Cysteamine was present on the serosal side (1 mM). Each value represents the mean ± S. D. (n > 5).

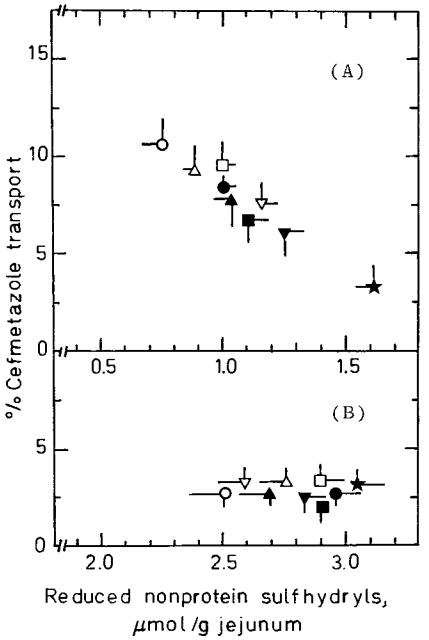


**Fig. 2** Relationship between the reduced nonprotein sulfhydryl levels in rat jejunal tissue and rat jejunal tissue permeability against cefmetazole. A. The effects of each adjuvant described in Fig. 1 were determined with the adjuvant present on the mucosal side (open symbols) or the serosal side (closed symbols). The symbol ★ represents the control values in the absence of adjuvant. B. Effect of cysteamine on the adjuvant action shown in A. Cysteamine was added to the serosal side (1 mM). Each value represents the mean ± S. D. (n > 5).

metazole, because the treatment with salicylate did not change the levels of total nonprotein sulfhydryl, including oxidized form, but decreased significantly the levels of RNS in intestinal tissue.

The above results suggest that the decrease of RNS concentration within the intestinal tissue may be responsible for the enhanced permeation of cefmetazole through the intestinal wall from the mucosal side to the serosal side. As shown in Figs. 1 A and 2 A, a good relationship between the concentration of RNS and the transport of cefmetazole was observed. The enhancing action of each adjuvant on cefmetazole transport was more pronounced in the colon compared to jejunum. When the surfactant sodium lauryl sulfate (1 %) was added to the mucosal side, cefmetazole transport 20 min after incubation was also increased by 18.4 ± 2.4 % (n = 4, $p < 0.001$ against control, Student's t-test), but without any significant change in the RNS concentration in the colonic tissue, (1.36 ± 0.11 μmol/g tissue, n = 8). However, sodium lauryl sulfate did not enhance cefmetazole transport (only 3.8 ± 1.1 %, n = 4) when added to the serosal side. These results suggest that the nonsurfactant adjuvants used in this study cause a decrease in the RNS tissue concentration which causes the observed change in membrane permeability.

Since the decreased RNS concentration closely correlates with the increased transport of cefmetazole through the intestinal barrier, it was examined whether cysteamine, one of the typical RNS, protects against the compromising effect of these adjuvants on the barrier function of mucosal membrane. As shown Figs. 1 B and 2 B, the addition of cysteamine to the serosal side suppressed the enhancing action of each adjuvant on the cefmetazole transport, and maintained the RNS concentration in each tissue at higher than normal levels. This result supports the hypothesis that RNS regulates at least in part the membrane permeability.

To examine the damage of intestinal tissue as a result of an adjuvant induced RNS depletion, the protein content in intestinal tissue was measured as an indicator of tissue damage. Protein content in both tissues was not affected by the adjuvants added at the concentration used above (Figs. 1 and 2). However, when the concentration of either DEEMM or DEM was further increased on the mucosal side, protein content in jejunal tissue (Fig. 3 A) and colonic tissue (Fig. 3 B) decreased abruptly. This observation suggests that treatment with DEEMM or DEM at high concentration caused a deterioration of membrane integrity when RNS concentration was reduced to less than 0.6 μmol/g tissue in jejunum and 0.5 μmol/g tissue in colonic tissue.

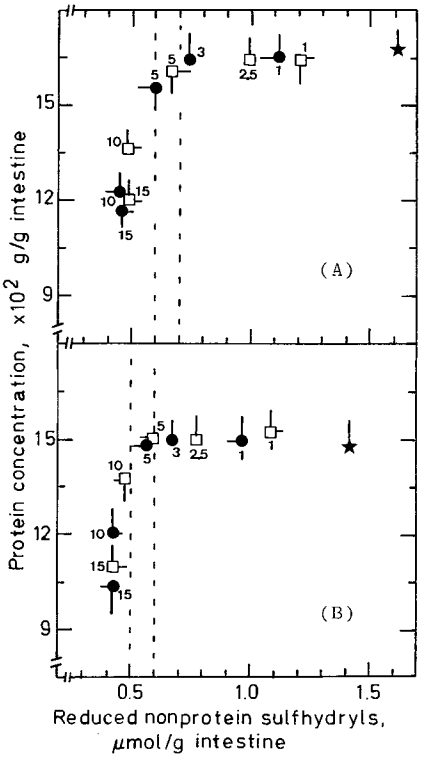


**Fig. 3** Relationship between reduced nonprotein sulfhydryl levels and protein concentrations in jejnum (A) and colon (B). The effects of diethyl maleate (●) or diethyl ethoxymethylene-malonate (□) are shown. The numbers next to each symbol indicate the concentration of DEM (mM) and DEEMM (mM) on the mucosal side. The symbols ★ represent the control value in the absence of adjuvant. Each value represents the mean ± S. D. (n > 4).

In conclusion, intestinal membrane permeability against lipophobic compounds may be regulated by the RNS

level in the intestinal tissue, and a significant decrease in RNS in mucosal cells can result in disruption of the intestinal membrane integrity.

**Acknowledgement**

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# Change of Phase-Solubility Behavior by Gamma-Cyclodextrin Derivatization

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**Abstract:** The effect of γ-cyclodextrin and its four derivatives on the solubility of progesterone in phosphate buffer pH 7.4 was investigated. γ-Cyclodextrin forms a complex precipitating from solution at low cyclodextrin concentrations. No precipitation of complexes was observed with the γ-cyclodextrin derivatives. This change in phase-solubility behavior is probably due to low crystallization tendencies of the derivatives.

The application of cyclodextrin complexes in the formulation of pharmaceutical preparations is usually limited to the use in solid formulations (1) because of the low aqueous solubility of β-cyclodextrin (2). On the other hand, both β-cyclodextrin as well as the highly water soluble γ-cyclodextrin often form complexes with limited aqueous solubility, thus resulting in solubility curves of the type $B_S$ (3). For example, γ-cyclodextrin forms $B_S$ type solubility curves with a large number of steroids and benzodiazepines (4, 5), which imposes serious limitations towards the use of γ-cyclodextrin in the formulation of liquid preparations.

We have previously shown (6) that alkylation of β-cyclodextrin with different substituents results not only in a better aqueous solubility of the derivatives compared with the parent compound, but also changes the type of solubility curves from the $B_S$-type with β-cyclodextrin to the A-type curve with the derivatives.

In the present paper, the effect of four different γ-cyclodextrin derivatives on the solubility of progesteron is examined. Derivatives of γ-cyclodextrin may be useful for the solubilization of drugs that form $B_S$-type solubility curves with non-substituted γ-cyclodextrin.

## Materials and Methods

γ-Cyclodextrin derivatives (Table I) were kindly donated by Kalle AG (Wiesbaden, FRG) and dried in vacuo before use. γ-Cyclodextrin was purchased from Lehmann & Voss (Hamburg, FRG). Progesterone (Merck, Darmstadt, FRG) was used in pharmaceutical grade without any further purification. All other materials and solvents were of analytical reagent grade.

**Table I.** γ-Cyclodextrin derivatives++

| Substituent | R | DS+ |
|---|---|---|
| Methyl- | $-CH_3$ | 1.49 |
| Hydroxyethyl- | $-CH_2-CH_2-OH$ | 0.77 |
| Hydroxypropyl- | $-CH_2-CH(OH)-CH_3$ | 0.66 |
| Carboxymethyl- | $-CH_2-COO\,Na$ | 0.86 |

+degree of substitution (average number of substituents per glucose subunit).
++For a detailed description of the γ-cyclodextrin derivatives, see (6, 7).

Solubility studies were carried out according to the methods of Higuchi and Connors (3). Excess amounts of progesterone were added to solutions containing various concentrations of cyclodextrins in phosphate buffer pH 7.4 (Ph. Eur.) and were shaken at 25 ± 0.5°C in the dark for one week. An aliquot of the suspensions was then centrifuged and pipetted through a 0.45 μm membranous filter.

An HPLC method was used for the quantitation of progesterone in the resulting solutions. The separation utilized a Shandon ODS Hypersil RP – 18 column (5 μm in 5 mm × 25 cm, Shandon, Runcorn, GB) with acetonitrile – water (70:30) as the mobile phase. The eluent was monitored spectrophotometrically at 240 nm. Progesterone was quantitated by measuring peak areas and comparing the areas with that of known amounts of extern standards.

The powder X-ray diffraction patterns were taken by a Diffrac 11 diffractometer (Siemens AG, München, FRG) after a three month storage of the dry substances at room temperature.

## Results and Discussion

Figure 1 shows the results of the solubility studies with γ-cyclodextrin and the four γ-cyclodextrin derivatives. With γ-cyclodextrin, a solubility curve of the $B_S$-type was obtained. The complex reaches its maximum solubility at a rather low cyclodextrin concentration (0.4 % w/v). A further addition of γ-cyclodextrin results in precipitation of the complex from solution and subsequently in a decrease of progesterone concentration (4).

On the other hand, with all γ-cyclodextrin derivatives solubility curves of the $A_L$-type were obtained. However, the slope of the solubility curves obtained with the derivatives is significantly smaller than that obtained with

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