Rectal Delivery of Antiinflammatory Drugs. II.[1] The Influence of Basic Amino Acid Salts on Rectal Absorption of Diclofenac[2]

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Rectal absorption of diclofenac (DC) and its salts with sodium (DCNa), L-arginine (DC-Arg), L-lysine (DC-Lys), and L-histidine (DC-His) was studied in dogs, rabbits and rats. Plasma levels of unchanged DC were measured by high performance liquid chromatography. Both the bioavailability and the plasma peak level of DC-Lys were the largest among the salts studied. The bioavailability of DCNa on oral administration in terms of plasma levels of DC was observed to be much larger than after rectal administration of DC but it was of the same order as those of rectal administration of DCNa, DC-Arg, and DC-His. The extents of bioavailabilty in rabbits and in rats were similar, but considerably better than that in dogs. The irritative effects of DC salts on the rectal mucosa in rats were studied by gross and light microscopic examinations following the administration of suspensions in 1% methyl cellulose solution. DC-Arg showed the weakest irritation of the rectal mucosa, and the irritation caused by 20% suspension was equivalent to that of 5% suspension of DCNa. The protective effects of counter ions against the histological damage caused by DC to the rectal mucosa increased in the order of sodium>lysine> arginine. From these results, it appears that the basic amino acid salts of DC may be useful as rectal delivery preparations for clinical use.

Keywords——diclofenac; arginine; lysine; histidine; rectal absorption; histology; suppository; HPLC-analysis; species difference; dissolution test

In the previous paper,[1] it was demonstrated that some nonesteroid antiinflammatory (NSAI) drugs showed strong promotting effects on the rectal absorption of sodium salts of ampicillin and cephalothin, which were unable to permeate readily through the rectal membrane without NSAI drugs as a result of their poor lipid affinity. The promoting efficacy of NSAI drugs was linearly correlated with their antiinflammatory activity against carragenan-induced paw edema. Thus, a possible irritative effect on the rectal mucosa was suggested to be responsible for the enhanced permeation of the antibiotics through the rectal membrane. To avoid mucosal damage, a combination of rectal with the oral administration was recommended.[3]

Various hypotheses have been proposed to explain the lesion formation in resonse to the oral administration of NSAI drugs. Examples include local irritation,[4] the inhibition of enzymatic reactions,[5] and the uncoupling of oxidative phosphorylation.[6] The mucosal irritation occurred not only on oral administration but also on rectal administration of NSAI drugs.[7]

It has been well established that some essential amino acids can significantly prevent aspirin-induced gastric mucosal damage.[8] Recently, Lim et al.[9] reported that methionine and histidine reduced the damage to the gastric mucosa induced by the oral administration of aspirin. Fujisawa et al.[10] also reported that pain induced by the intramuscular injection of β-lactam antibiotics could be reduced by their administration in the form of salts of basic amino acids without unfavorable effects on the bioavailability.

Thus, we have started to study the application of basic amino acids to rectal delivery preparations of acidic NSAI drugs in the hope of reducing possible mucosal damage at the administration site and improving the bioavailability.

In the present paper, diclofenac, 2-[(2,6-dichlorophenyl) amino]benzeneacetic acid (DC), was used because, to our knowledge, no attempt has yet been made to administer it rectally. To study the change of rectal mucosa, gross and light microscopic examination were performed.

Experimental

Materials——Sodium salt of DC obtained from Nippon Bulk Yakuhin Co., Ltd. was recrystallized twice from water (mp 283—285°C). DC was prepared from DCNa. The aqueous solution of DCNa was acidified with 1 N HCl and extracted with ether. The ether layer was evaporated to dryness under reduced pressure. Double recrystallization from a mixture of equal volumes of ethyl ether and petroleum ether gave needles of DC (mp 156—158°C). L-Arginine (Arg), L-lysine (Lys), and L-histidine (His) were obtained from Wako Pure Chemical Industries Co., Ltd. and were used without further purification. Methyl cellulose of 4000 cps was obtained from Tokyo Kasei Industries Co., Ltd. All other reagents and solvents were of reagent grade and were used without further purification.

Preparation of Amino Acid Salts of DC——An aqueous solution of Arg (1.92 g, 0.011 mol) dissolved in 10 ml of water was added to a suspension of 2.82 g (0.01 mol) of DC in a mixture of methanol (50 ml) and water (50 ml) at room temperature under stirring. The mixture was stirred for 1 h, a small amount of precipitate was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was crystallized from water giving needles of DC-Arg (mp 175—178°C) in good yield.

Similarly, DC-Lys and DC-His were obtained (mp 141—144°C and 245—248°C, respectively).

Preparation of Suppositories——The suppository base used was Witepsol H-15 (Dynamit Novel A.G. Chemische Witten, G.F.R.). Suppositories for the experiments in dogs and rabbits were prepared in the same manner as described in the previous poper[1] so as to contain 50 mg of drugs in terms of DCNa per gram. For the study in rats, the molten suppository was poured into a silicone-rubber tube (inner diameter 2.5 mm) at 30—32°C. The suppositories were cooled at 10°C and kept at 4°C until use. The drug content in suppositories was determined by the high-performance liquid chromatographic method (HPLC) and found to be $\pm 0.1\%$ of the stated amount.[1]

In Vitro **Dissolution Test**——The dissolution of a drug from the suppositories was measured following the method described previously.[1]

In Vivo **Absorption Study**——Adult male beagle dogs weighing 10—13 kg, adult male rabbits weighing 2.3—2.5 kg, and adult male Wistar rats weighing 230—250 g were used. Animals were fasted for 24 h before the experiments, but water was given freely. The dose of DC salts was fixed at 10 mg/kg DC-equivalent in all experiments, unless otherwise stated. The concentration of NSAI drugs in suppositories was fixed at 5% (w/w) DC-equivalent in the absorption study. The procedures of suppository administration, plasma sample collection, and analysis were as reported previously.[1]

Histological and Pathological Study——Male Wistar rats weighing 230—250 g were used. The rats were fasted for 24 h prior to the experiments but water was given freely. The drug suspended in 1% methyl cellulose (MC) solution was injected into the rectum with a 0.5-ml syringe. The concentrations of drugs administered were 5% (5%-Rp) and 20% (20%-Rp) in terms of DCNa. They were designated in the following experiments as 5%-Rp and 20%-Rp, respectively.

Before rectal administration, the bowel contents in the lower rectum were evacuated by pressing down on the abdomen. A 50 mg portion of the suspension was administered into the rectum and the anus was lightly moistened with water and glued shut with Alon Alpha to prevent leakage of the suspension. At a designated time of 1 or 2 h after the administration, the rat was lightly anesthetized with ether and sacrificed by decapitation. The rectum and anus were removed as one segment with a length of approximately 2.5 cm. Following a conventional method,[11] the tissues were fixed with 10% formaldehyde solution, dehydrated, embedded in paraffin, and sectioned at 3 mm intervals with several slices of 5 μ thick at each section. Each slice was stained with hematoxylin–eosin and examined under a light microscope.

Results and Discussion

Rectal Absorption of Diclofenac and Its Salts

The chronological changes of plasma concentration of DC in dogs following rectal administration of Witepsol suppositories containing DC or its salts are presented in Fig. 1. Similarly, the results on apparent bioavailability and some biopharmaceutical parameters of DC and its salts are presented in Table I. For comparision the results of oral administration of DCNa in dogs are also presented in the figure and table. The values of bioavailability were routinely obtained from the ratio of the values of AUC of 0—240 min following rectal or oral administration to those following intravenous administration of DCNa at the same dose. Riess *et al.*[12] reported that the taurine conjugate and acyl-glucuronide were formed as metabolites of DC
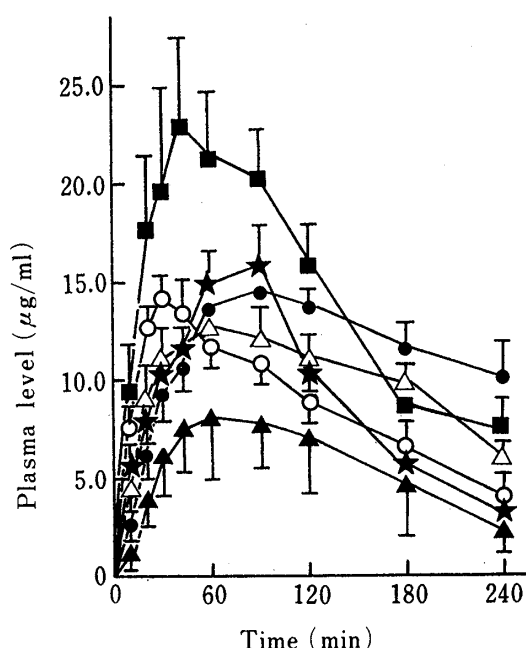
Fig. 1. The Plasma Concentration of Unchanged Diclofenac after the Rectal Administration of Diclofenac and Four Diclofenac Salts in Dogs

-▲- : DC, -○- : DCNa, -■- : DC-Lys, -●- : DC-Arg, -△- : DC-His, -★- : DCN $(p. o,)$ Each point represents the mean±S.D. of five animals. Dosage was 10 mg/kg in terms of DCNa.

in dog urine. Tsuchiya *et al.*[13] also studied the disposition of DC and its metabolites and suggested the enterohepatic circulation of DC from the results of intravenous administration of DC and intraduodenal administration of the glucuronide in dogs. Thus, the disposition of DC may be influenced by the route of administration.

In the present experiments, only the unchanged DC present in plasma was determined because the aim of this work was a comparative study of the effects of basic amino acids on the rectal absorption of DC, and only the unchanged species of DC is considered to be clinically effective.[14]

The Values of bioavailability and peak plasma level, C_{max}, of DC-Lys were the largest among those of the salts studied and were significantly larger than those following oral administration of DCNa. From the results of preliminary experiments, the gastrointestinal absorption of DC or DCNa was not influenced by the coadministration of basic amino acids. It was interesting that the rectal absorption of DCNa in terms of AUC values of DC in plasma was comparable to the oral absorption, suggesting equivalent clinical effectiveness for both administration routes. Thus, the lysine salt is considered to be adequate for rectal delivery preparations of DC.

The rectal absorption of DC salts in terms of AUC of unchanged DC was found to be linearly correlated with the dose suggesting that the absorption occurred by passive transport (Fig. 2).

To study the differences of rectal absorption between experimental animal species, similar experiments were performed in rats and rabbits at the same dose of 10 mg/kg (Fig. 3). It is necessary to take into consideration the differences in metabolism in the living body between

TABLE I. The Plasma Level of Unchanged Diclofenac after the Rectal Administration of Diclofenac and Its Salts in Dogs (10 mg/kg equivalent for DCNa)

Drug administered		Peak time (min)	Peak level (μg/ml)	AUC_{0-240}[c] (min·μg/ml)	Bio.[d] (%)	n
Route	Salt					
Rectal	DC	60	7.9 ± 3.5[a]	1400 ± 707[a]	26 ± 14[a]	5
	DCNa	30	13.8 ± 0.6	2478 ± 279	46 ± 5	5
	DC-Lys	40	23.5 ± 4.4[a,b]	3414 ± 304[a,b]	63 ± 6[a,b]	5
	DC-Arg	90	14.7 ± 4.1	2590 ± 107	48 ± 3	5
	DC-His	60	13.7 ± 6.6	2239 ± 468	41 ± 9	5
Oral	DCNa	60	13.0 ± 2.3	2045 ± 935	38 ± 17	5

a) $p < 0.05$ Significant difference from DCNa (rectal route).
b) $p < 0.05$ Significant difference from DCNa (oral route).
c) Obtained from Fig. 1 (0—240 min).
d) Bioavailability.
The values are means ± S.D.
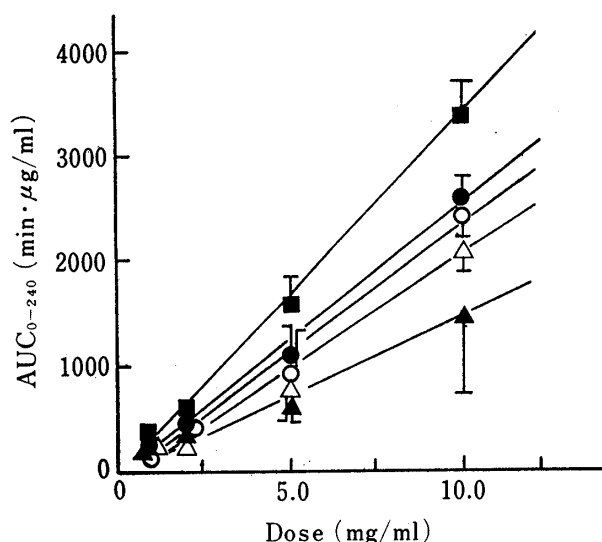The oral dosage form was hard gelatine capsules.

Fig. 2. The Relationship between Dose and AUC_{0-240} after the Rectal Administration of Various Diclofenac Salts in Dogs

-○- : DCNa, -■- : DC-Lys, -●- : DC-Arg, -△- : DC-His.
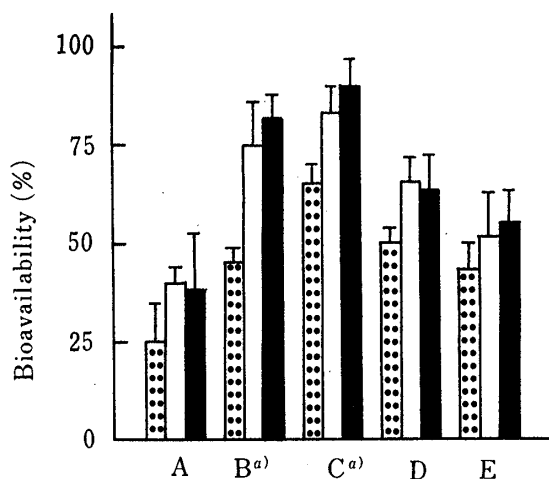Each point represents the mean ± S.D. of five animals.

Fig. 3. Comparison of Bioavailability between Species after the Rectal Administration of Various Diclofenac Preparations

A: DC, B: DCNa, C:.DC-Lys, D: DC-Arg, E: DC-His
▨ : dogs, ·☐ : rabbits, ■ : rats.
Each histogram represents the mean ± S.D. of five or six animals. Dose was 10 mg/kg in terms of DCNa.
a) Significant difference between dogs and rabbits or rats ($p < 0.05$).

animal species, but, in general, the absorption in rats and rabbits is considered to be greater than that in dogs. The order of absorption among DC and its salts was consistent in each animal species.

The permeation of drugs through the rectal membrane involves many complicated factors, but it may be essential that drugs incorporated into a suppository base should be released
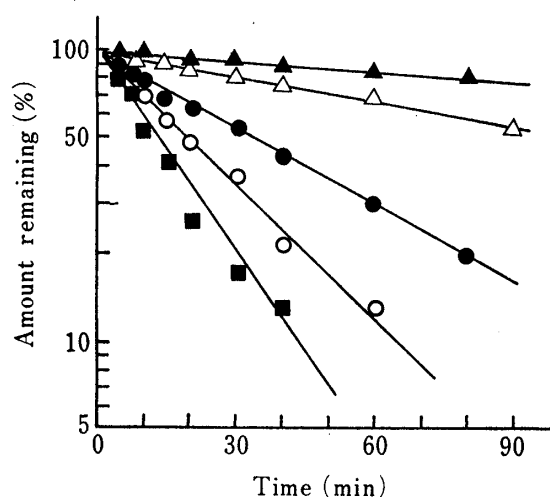

Fig. 4. Dissolution of Diclofenac and Its Salts from Suppositories

-▲- : DC, -○- : DCNa, -■- : DC-Lys, -●- : DC-Arg, -△- : DC-His.
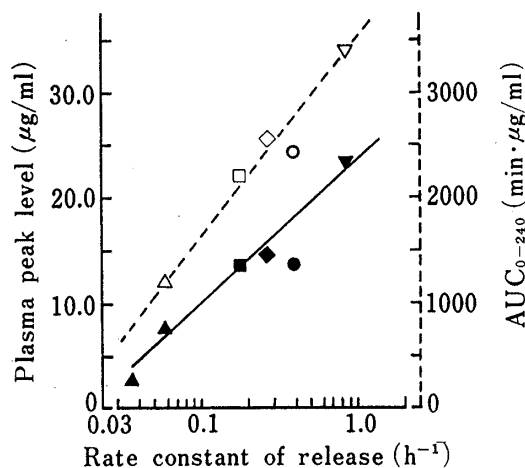

Fig. 5. Relationship between the Dissolution Rate Constant and the Plasma Peak Level or AUC_{0-240} after the Rectal Administration of Diclofenac and Its Salts in Dogs

Plasma peak level: -▲- : DC, -●- : DCNa, -▼- : DC-Lys, -◆-: DC-Arg, -■-: DC-His.
AUC_{0-240} : ···△··· : DC, ···○··· : DCNa, ···▽··· : DC-Lys, ···◇···: DC-Arg, ···☐··· : DC-His.

from the base into the rectal fluid at the early stage after administration. Fig. 4 shows the results of *in vitro* dissolution experiments with DC and its salts from suppositories. All drugs were released from suppositiories according to apparent first-order kinetics. The poor absorptivity of DC may be partly explained on the basis of its limited release within the experimental period. To clarify the effects of release on the rectal absorption, the rate constants of release were obtained from the slopes of the plots in Fig. 4. Linear correlations were observed between the logarithm of the rate constants and the peak plasma levels or the values of AUC with correlation coefficients of more than 0.9 (Fig. 5). Thus, the release and dissolution processes are considered to be important for the rectal absorption of DC salts.

Histological and Pathological Observation of Rectal Mucosa after Rectal Administration of Diclofenac Salts

Serious gastrointestinal lesions and ulceration have been frequently encountered after the ingestion of NSAI drugs.[15] Similar irritation of the rectal membrane was reported after the rectal administration of NSAI drugs.[7] Therefore, to evaluate the clinical applicability of the present results, a histological examination of rectal mucosa was required. To avoid the influence of the process of dissolution of drugs from lipophilic suppositories, suspensions in 1% MC solution were used and the degree of irritation was determined by gross and microscopic examinations.

TABLE II. Histological Findings in the Rectal Membrane after the Administration of Several Kinds of Suppositories in Rats

Observation	Normal	1%MC	DCNa 5%-Rp 1 h	DCNa 5%-Rp 2 h	DCNa 20%-Rp 2 h	DC-Lys 5%-Rp 1 h	DC-Lys 5%-Rp 2 h	DC-Lys 20%-Rp 1 h	DC-Lys 20%-Rp 2 h	DC-Arg 5%-Rp 1 h	DC-Arg 5%-Rp 2 h	DC-Arg 20%-Rp 1 h	DC-Arg 20%-Rp 2 h
Mucosa													
DEC	−	±	+ +	+ +	+ +	±	±	±	±	±	±	±	±
ND	−	−	+	+	+ +	±	±	+	±	±	−	±	±
HY	−	−	+	+	+	−	−	±	±	−	−	−	+
HE	−	−	+	+	+	−	−	±	−	−	−	−	±
ICI	−	−	+	+	+	−	−	+	±	−	−	±	−
Submucosa													
Edema	−	−	±	+ +	+ +	−	−	±	±	−	−	±	±
HE	−	−	−	+	±	−	−	−	−	−	−	−	−
ICI	−	−	−	+	+	−	−	±	±	−	−	−	−
n	3	2	4	4	4	4	4	4	4	4	4	4	4

DEC: deficiency of epithelial cells, ND: necrosis of degradation,
HY: hyperemia, HE: hemorrhage, ICI: inflammatory cell infiltration.
+ +: severe irritation, +: moderate irritation, ±: slight irritation,
−: no irritation.

Histological observation was performed at 1 or 2 h after the administration of 5%-Rp or 20%-Rp and the changes in mucosa and submucosa were scored into four classes according to the method of Ito[16] with some modification (Table II). In general, no histological change was observed after the administration of 1% MC alone, but DC salts had some irritative effects on tissues and DCNa was the strongest irritant among the salts studied.

The results of microscopic observation are presented in Figs. 6—9. In Fig. 6(A), photographs of untreated rectal mucosa are presented. They show normal and clear images of epithelial cells with crypts of Lieberkühn lined by goblet cells and principal cells. Fig. 6(B) shows micrographs taken after the administration of 1% MC alone. A slight deficiency of epithelial

cells at the luminal border was observed in many specimens but no change was observed at the crypt region or the submucosal layer. This observation suggests that the rectal mucosa is easily modified by the administration of 1% MC solution but that the change is not serious. Fig. 7 shows micrographs obtained 1 h after the administration of 5%-Rp of DCNa and 2 h after the administration of 20%-Rp. In the photographs, severe histological changes, such as deficiency of epithelial cells, necrotic degradation of the mucosa, hyperemia of the capillaries, hemorrhage, and inflammatory-cell infiltration into the submucosa, are distinctly observed. Edema with hemorrhage was also observed in the submucosal layer. From these results and the gross observations, it may be considered that such severe damage would require a fairly long period for recovery, and essentially rules out the clinical application of DCNa in rectal delivery preparations.

The results with DC–Lys 1 h after the administration of 5%-Rp and 2 h after the administration of 20%-Rp are presented in Fig. 8. The histological changes observed in these specimens were similar to those seen with DCNa, but the degree of damage was moderate or slight. Some inflammatory-cell infiltration was observed, but the degree was slight in comparison
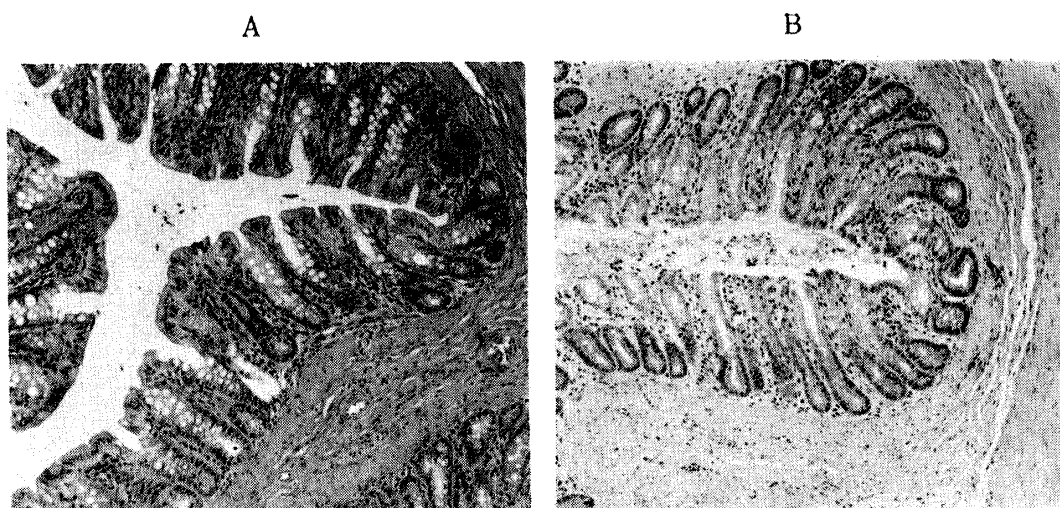
A   B

Fig. 6.   The Rectal Mucosa of Rats in Untreated (A) and 1%
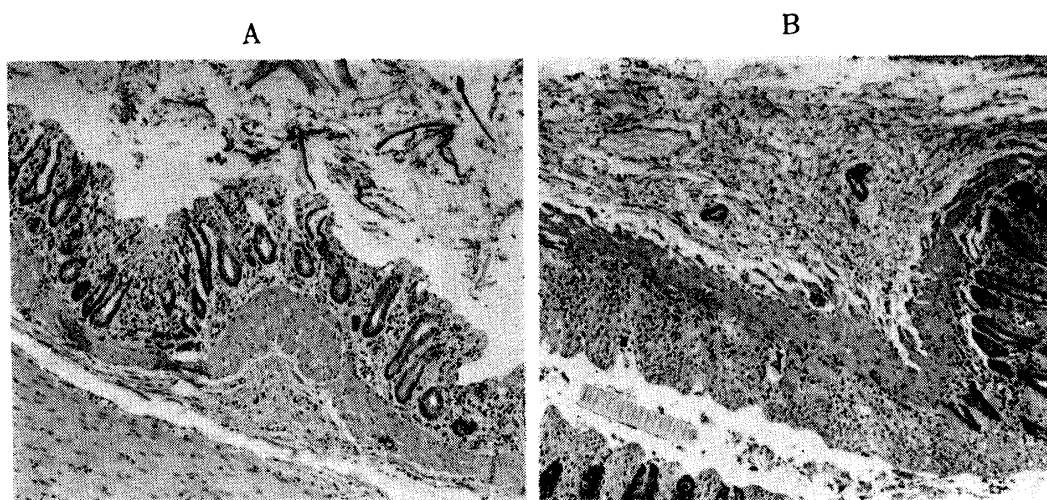MC-Treated (B) Controls (×25)

A   B

Fig. 7.   The Rectal Mucosa of Rats treated with 5%-Rp of DCNa
for 1 h (A) and with 20%-Rp of DCNa for 2 h (B) (×25)
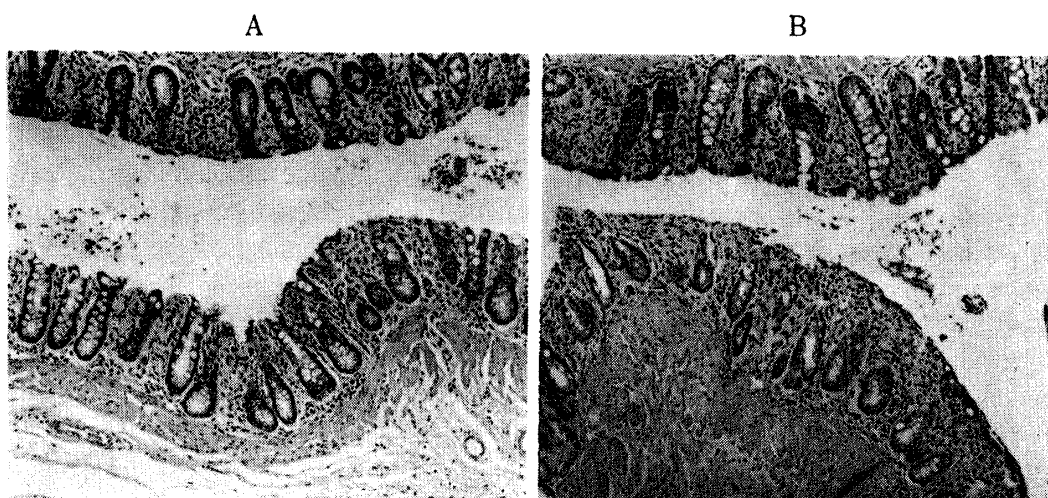
A   B

Fig. 8.   The Rectal Mucosa of Rats treated with 5%-Rp of DC–Lys
for 1 h (A) and with 20%-Rp of DC–Lys for 2 h (B) (×25)

A   B
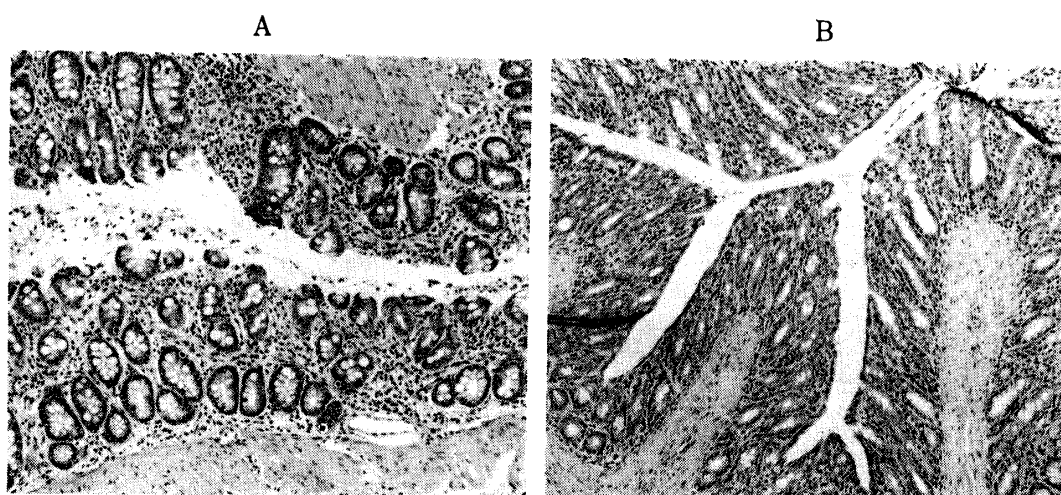

Fig. 9.   The Rectal Mucosa of Rats treated with 5%-Rp of DC–Arg
for 1 h (A) and with 20%-Rp of DC–Arg for 1 h (B) (×25)

with that cause by DCNa.   The irritative effect of 20%-Rp is considered to be equivalent to that of DCNa at 5%-Rp.

Fig. 9 shows photographs taken at 1 h after the administration of 5%-Rp of DC-Arg and at 2 h after the administration of 20%-Rp.   Minor changes, such as necrotic degradation at the mucosal surface, deficiency of epithelial cells, hyperemia of capillaries, inflammatory-cell infiltration into the submucosa, and edema at the submucosa, were observed, but their degree was slight in comparison with the changes caused by DCNa and DC–Lys.

Thus, the protective effects of counter ions on the histological damage caused by DC to the rectal mucosa increase in the following order: sodium≪L-lysine<L-arginine.   It is interesting that this order is not reflected in the results of rectal absorption of DC salts, suggesting an insignificant contribution of the irritative effects of DC salts to their rectal absorption. The absorption of DC salts from the rectum is considered to occur mainly by simple diffusion, subject to the lipid affinity of ionized species of DC, because DC (pK_a 4.0[17]) is largely ionized in the rectal fluid at pH 7.[18]   This view is supported by the large apparent partition coefficient, about 14 at 37°C, between n-octanol and phosphate buffer at pH 7; the value was not influenced by the addition of basic amino acids.

The standard dose of DCNa clinically used for oral administration is about 2 mg/kg.[19] In the present experiments, the doses of 5%-Rp and 20%-Rp were 5 and 20 times larger than the clinical oral dose. Thus, in view of the superior bioavailability of DC salts administered rectally as compared with oral administration of DCNa, the dose in rectal administration of DC salts can be reduced, and may give a sufficient pharmacological activity without causing detectable irritation of the rectal mucosa.

The results presented in this study indicate that basic amino acid salts of DC may be applicable as rectal delivery preparations for clinical purposes. Further work is in progress to clarify the mechanisms of protective action of basic amino acids against the irritative effects of DC.

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