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Research Article

Changes in Intestinal Mucosal Permeability Caused by Nonprotein Thiol Loss in Rats

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The barrier selectivity of the intestinal mucosal membrane permeability may be impaired in certain disease conditions. Membrane permeability was previously shown to be correlated with changes in nonprotein thiol in rat intestinal tissue by the everted sac method. In the present study, the mucosal effects of alloxan-induced diabetes and chronic alcohol administration to intact rats, as well as pretreatment with diethyl maleate, ethanol, and salicylate, were investigated. In each case, a drop of mucosal nonprotein thiol was associated with an increased absorption of cefoxitin, cefmetazole, and phenol red, hydrophilic compounds that are poorly absorbed through intact membrane, and with a decreased absorption of L-phenylalanine. The effect of nonprotein thiol loss on rectal absorption of cefoxitin, cefmetazole, and phenol red was greater than that on the small intestinal absorption. The increase in phenol red absorption by diethyl maleate in the *in vitro* everted sac method correlated with Ca^{2+} release from the intestinal mucosa, which was induced by nonprotein thiol loss. Resistance to the effect of nonprotein thiol loss on Ca^{2+} homeostasis was greater in rat ileum than in rat colon (including rectum). The administration of cysteamine as an exogenous nonprotein thiol restored nonprotein thiol levels in the mucosa along with the barrier function of the intestinal mucosa to the absorption of cefoxitin, cefmetazole, and phenol red. In contrast, the transport of L-phenylalanine in the small intestinal mucosa was not restored by cysteamine treatment.

KEY WORDS: mucosal membrane permeability; intestine; nonprotein thiol; Ca^{2+} release; hydrophilic compounds.

INTRODUCTION

The intestinal membrane barrier represents a vitally important interface with our environment since drugs and nutrients must be absorbed across the gut wall. Many hydrophilic compounds, however, are not readily taken up from the intestinal lumen. A better understanding of how mucosal membranes regulate permeation will aid in efforts to optimize nutrient and drug absorption, especially under various disease states. Depletion of nonprotein thiols by treatment with diethyl maleate, ethanol, or salicylate increased the transport of cefmetazole (a hydrophilic compound) from the mucosal side to the serosal side, in the *in vitro* rat everted intestinal sacs (1). Further, glutathione, a major endogenous nonprotein thiol, was shown to maintain cell integrities (2).

In the present study, we examined the regulatory influence of nonprotein thiols on the transport of hydrophilic compounds, including cefoxitin, cefmetazole, phenol red, and L-phenylalanine, across rat rectal and small intestinal mucosa in diabetic and alcohol-treated rats, by an *in situ* intestinal loop method. We also demonstrate that Ca^{2+} release from the intestinal mucosa after nonprotein thiol loss increased intestinal mucosa permeability to phenol red, by an *in vitro* everted intestinal sac method.

MATERIALS AND METHODS

Materials

Diethyl maleate, ethanol, sodium salicylate, cysteamine, L-phenylalanine, vanadium pentoxide, and arzenazo III were purchased from Sigma Chemical Inc. (St. Louis, Mo.). Vanadate was prepared by dissolving 1 mmol of vanadium pentoxide in 1 ml of 1 *N* NaOH. Cefoxitin and cefmetazole were supplied from Merck Sharp and Dome (Rahway, N.J.) and Sankyo Co. (Tokyo), respectively. Phenol red was purchased from Nakarai Chemical Co. (Kyoto, Japan). Other reagents used were of analytical grade.

Animals

Male Sprague–Dawley rats (200 to 250 g) were used for

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the *in situ* intestinal loop study. Diabetes was induced by the administration of alloxan (100 mg/kg, subcutaneously) to rats. Serum glucose concentration as an indicator of diabetic state was measured by an assay kit (diagnostic kit No. 510, Sigma) which is based on the enzymatic method. Alcoholic rats were obtained by giving a 5% ethanol solution to rats for 30 days or more. Serum γ-glutamyltransferase activity as an indicator of disease state was measured with an assay kit (diagnostic kit No. 545, Sigma) according to Posalki and Rau (3).

Male Wistar rats (230 to 300 g) were used for the *in vitro* everted intestinal sac study.

Rats were fasted for 16 hr prior to the experiments and all animal studies were carried out between 9 and 11 AM.

In Situ Intestinal Loop Study

Absorption of compounds from the intestinal lumen was determined as the disappearance rate of test compounds from rectal and small intestinal ligated loops by the following equation:

$$\text{disappearance rate } (\mu\text{mol/hr/g tissue}) = (D - R)/[D_x(\text{period})(\text{tissue wet weight of intestinal loop})]$$

Here D and R represent the administered amounts and remaining amounts of test compound in the loop, respectively. The rectal segment including lower colon was ligated 4 cm from the anus (anal aperture also tied) and a 4-cm small intestinal segment was ligated with the proximal end 20 cm from the bile duct. Thirty minutes after the administration of 0.25 ml sodium phosphate buffer (0.1 *M*, pH 7.0) containing each test compound, the ligated intestinal loops were excised, and the intestinal lumen was rinsed with 8 ml saline. The combined rinse solution was adjusted to 10 ml. Administration into the loop of 0.25 ml buffer containing a SH-modifying agent such as diethyl maleate, ethanol, or salicylate, which decreases nonprotein thiol concentrations in intestinal tissue (1), was performed 15 min before the administration of test compounds.

Cysteamine was given intramuscularly at a dose of 50 mg/kg 30 min before the absorption experiments.

In a separate study, the nonprotein sulfhydryls were measured in the intestinal mucosa 15 min after the administration of each SH-modifying agent into the intestinal loop. After rinsing the excised loop tissue, the mucosal lining of each loop was scraped off with a glass knife and then homogenized with 4 vol of ice-cold 0.1 *M* phosphate buffer (pH 7.0). Nonprotein thiols and total nonprotein sulfhydryls in aliquots of the homogenate were measured according to the method of Ellman (4), using glutathione as the standard compound as suggested by Szabo *et al.* (5). Mucosal protein concentration was measured by the method of Lowry *et al.* (6), while homogenates were dialyzed against chilled water for 24 hr before assay to avoid interference by salicylate.

In Vitro Everted Intestinal Sac Study

Approximately 7 cm of colon (including rectum) and ileum was removed from rats. For the transport study of phenol red, the *in vitro* everted intestinal sac method was employed (7). Krebs–Ringer's solution and Ca^{2+}-free Krebs–Ringer's solution were used as the media and are referred to as Ca^{2+} medium and Ca^{2+}-free medium, respectively. The mucosal medium was saturated with oxygen:carbon dioxide (95:5) during the experiments.

The everted intestinal sac containing 1 ml medium was immersed into 10 ml medium containing phenol red (0.5 mg/ml) at 37°C. After 15, 30, and 60 min, serosal medium was collected to determine the amounts of phenol red transported. Transport of phenol red was represented as the clearance rate of phenol red, CL_{pr}, through the intestinal sac from the mucosal side to the serosal side with the following equation.

CL_{pr} = (cumulative amounts of phenol red in serosal medium)/[(initial concentration of phenol red in mucosal medium) (period) (wet weight of intestinal sac)]

In separate experiments using the same method without phenol red, the mucosal medium was collected to determine Ca^{2+} release according to the method described by Tone *et al.* (8) with arzenazo III using a Shimadzu multipurpose spectrophotometer (MPS-2000). Also, the intestinal mucosal lining was scraped off to determine nonprotein thiol in the mucosa by the method described earlier.

Assays of Test Compounds

Assay of cefmetazole (9), cefoxitin (9), and L-phenylalanine (10) was performed according to the high-pressure liquid chromatographic methods, described previously. Assay of phenol red was performed by a spectrophotometric method (11).

RESULTS

In Situ Intestinal Loop Study

The intestinal absorption of cefoxitin, cefmetazole, phenol red, and L-phenylalanine was studied in rats with diabetes or alcoholism or after pretreatment with SH-modifying agents. As shown in Table I, under each of the two disease conditions a decrease in nonprotein thiol concentrations was observed in small intestinal and rectal mucosae relative to controls. An increase in (at the rectum) and an increasing tendency to (at the small intestine) the absorption of cefoxitin, cefmetazole, and phenol red were observed in diabetic and alcoholic rats (Fig. 1), while the absorption of L-phenylalanine at the small intestine was decreased (Fig. 2).

To clarify the relationship between the concentration of nonprotein thiols and membrane permeability, the small intestinal and rectal segment was treated 15 min before the administration of test compounds with one of the SH-modifying agents. (The SH-modifying agent was not rinsed out before the administration of test compound.) Pretreatment with any of these agents resulted in enhanced rectal and small intestinal absorption of cefoxitin, cefmetazole, and phenol red, relative to the control (Fig. 1). However, the increase in their absorption from the rectal lumen was greater than that from the small intestinal lumen. Further, diethyl maleate, ethanol, and salicylate caused a significant decrease in the concentration of nonprotein thiols in the rat small intestinal and rectal mucosa, relative to the control (Table I). Of the total nonprotein sulfhydryls (i.e., total rep-

Table I. Concentrations of Nonprotein Thiol and Total Nonprotein Sulfhydryl in the Mucosa of Rat Small Intestine (S) and of Rectum (R) Including Lower Colon, Under the Disease Conditions or After Treatment with SH-Modifying Agents[a]

Condition (intestine)	Nonprotein sulfhydryls in mucosa (μmol/g protein)			
	No cystemaine treatment		Cysteamine treatment	
	Total	Thiol	Total	Thiol
Control (saline)				
S	12.9 ± 1.2	12.2 ± 1.1	13.2 ± 1.1	12.9 ± 1.4
R	10.9 ± 1.1	10.5 ± 0.8	11.9 ± 1.6	11.1 ± 1.1
Diabetic				
S	10.1 ± 1.9	9.2 ± 1.1*	12.2 ± 1.4	11.9 ± 0.9***
R	9.9 ± 1.4	8.4 ± 0.7*	11.3 ± 1.2	10.6 ± 0.9***
Alcoholic				
S	8.1 ± 1.2*	7.5 ± 0.8*	11.4 ± 1.7***	10.7 ± 0.9****
R	8.1 ± 0.6*	7.3 ± 1.0*	11.6 ± 1.3***	10.1 ± 1.0****
DEM				
S	3.4 ± 0.6**	3.3 ± 0.7**	6.8 ± 0.7****	6.4 ± 0.9****
R	3.2 ± 0.4**	3.1 ± 0.5**	6.2 ± 0.6****	6.1 ± 0.4****
Ethanol				
S	3.5 ± 0.6**	3.1 ± 0.8**	8.2 ± 1.1****	8.0 ± 0.9****
R	2.4 ± 0.4**	2.2 ± 0.9**	8.9 ± 1.2****	8.3 ± 1.3****
Salicylate				
S	12.2 ± 1.1	9.0 ± 1.0*	14.2 ± 1.9	11.8 ± 1.3***
R	10.1 ± 1.0	7.9 ± 1.4*	13.9 ± 1.6	10.6 ± 0.9***

[a] Buffer (0.25 ml) containing either no agent, 5 m*M* diethyl maleate, 10% ethanol, or 200 m*M* sodium salicylate was administered into each loop of small intestine and colon 15 min before assays. Cysteamine was administered intramuscularly at a dose of 50 mg/kg 30 min before assay. Each value represents the mean ± SD (N = 4). Diabetic rats used had plasma glucose levels of 420 to 480 mg/100 ml. Alcoholic rats used had serum glutamyltransferase activity of over 200 U/liter (14 ± 4 U/liter in normal rats; N = 4).

* $P < 0.1$ versus control.

** $P < 0.05$ versus control.

*** $P < 0.1$ versus no cysteamine treatment.

**** $P < 0.05$ versus no cysteamine treatment.

resenting nonprotein thiols and oxidized sulfhydryls combined) remaining in the rectal and small intestinal mucosa, 85% or more was in the thiol state under all conditions, i.e., after treatment with diethyl maleate and ethanol, in control and diabetic rats. The administration of salicylate, on the other hand, did not cause a drop in the total nonprotein sulfhydryls. However, the percentage of the thiol form was substantially decreased (Table I).

The decrease in nonprotein thiols had the opposite effect on L-phenylalanine absorption across the small intestinal mucosal membrane, i.e., the absorption of L-phenylalanine decreased (Fig. 2).

Intramuscular injection of cysteamine (50 mg/kg) to either diabetic or alcoholic rats restored the nonprotein thiols in the intestinal mucosa (Table I). The cysteamine treatment also suppressed the intestinal absorption of cefoxitine, cefmetazole, and phenol red, which was increased under the disease conditions, of diabetes and alcoholism (Fig. 1). However, it did not restore the decreased absorption of L-phenylalanine under the disease conditions (Fig. 2). The cysteamine treatment suppressed the increased absorption of cefoxitin, cefmetazole, and phenol red caused by diethyl maleate, ethanol, or salicylate, along with the increase in nonprotein thiols of the intestinal mucosa (Table I). In contrast, the cysteamine treatment did not restore the absorption of L-phenylalanine, which was decreased by treatment with diethyl maleate, ethanol, or salicylate (Fig. 2).

In Vitro Everted Intestinal Sac Study

Diethyl maleate (1m*M*) in the mucosal medium increased CL_{pr} through the colonic sac but did not increase CL_{pr} through the ileal sac, after 30 min of incubation with Ca^{2+} medium (Fig. 3A). The addition of vanadate to media on both sides increased CL_{pr} significantly in the presence of diethyl maleate, in spite of the lack of effect caused by vanadate alone. For the first 15 min, CL_{pr} through intestinal sacs was not increased by the presence of diethyl maleate alone or both diethyl maleate and vanadate (Fig. 3A), in spite of a significant nonprotein thiol loss (Table II). However, after 60 min, CL_{pr} through the ileal and colonic sacs increased even in the presence of diethyl maleate alone.

When the Ca^{2+}-free medium was used (Fig. 3B), CL_{pr} after 30 and 60 min was increased significantly in both colonic and ileal sacs in the presence of diethyl maleate, while CL_{pr} was not increased after 15 min in spite of a significant nonprotein thiol loss (Table II). The nonprotein thiol loss caused by the presence of diethyl maleate occurred rapidly,

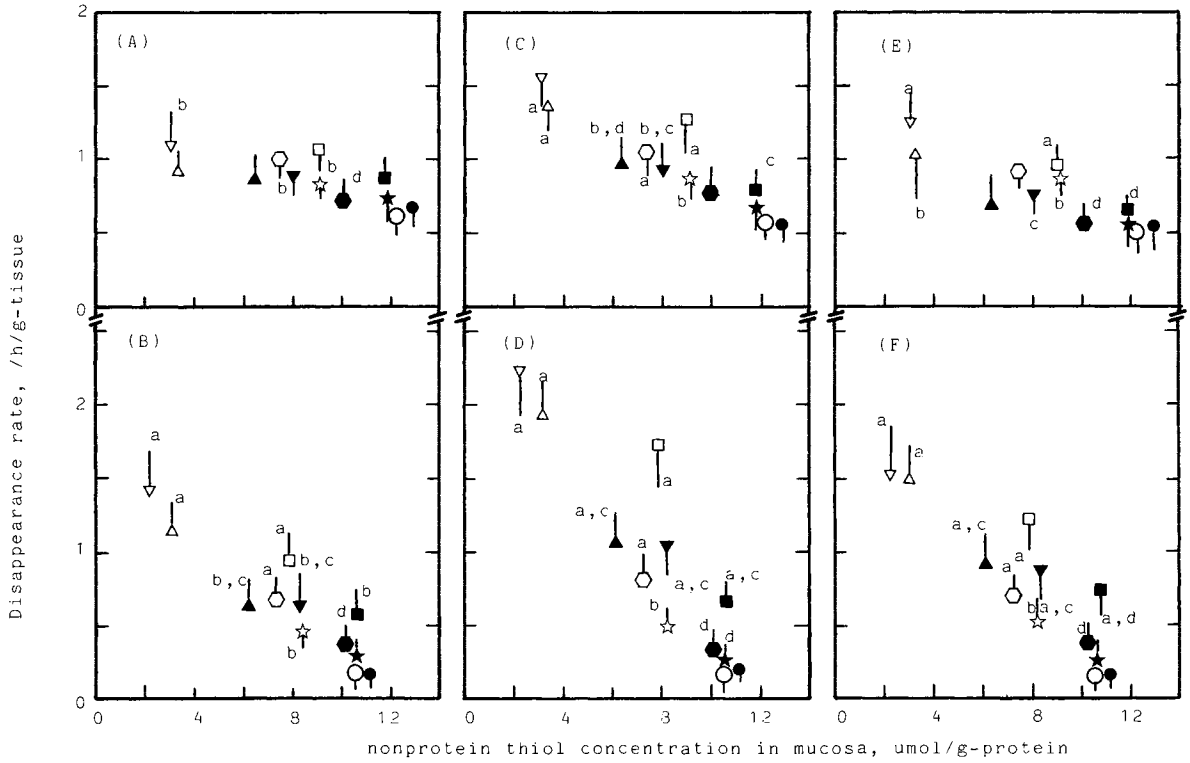

Fig. 1. Disappearance rate of phenol red (A, B), cefoxitin (C, D), and cefmetazole (E, F) from the small intestinal loop (A, C, and E) or rectal loop (B, D, and F) after the administration of each test compound in rats with diabetes (☆) or alcoholism (○) or after pretreatment with no agent (control; ○), diethyl maleate (5 m*M* in administered solution; △), ethanol (10%; ▽), and salicylate (200 m*M*; □). Data represent the disappearance rate of test compound against nonprotein thiol levels in the mucosa, which were obtained from Table I. The effect of cysteamine treatment (50 mg/kg, im, 30 min before absorption study) on the disappearance rate of test compounds is represented by filled symbols. The dose of each test compound was 5 mg/loop for cefoxitin and cefmetazole and 1 mg/loop for phenol red. Each value represent the mean ± SD ($N = 4$). (a) $P < 0.01$ versus control (Student's t test); (b) $P < 0.05$ versus control; (c) $P < 0.05$ versus no cysteamine treatment; (d) $P < 0.1$ versus no cysteamine treatment.

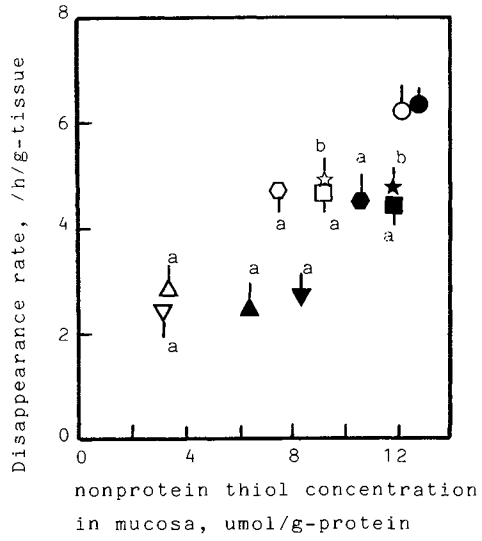

Fig. 2. Disappearance rate of L-phenylalanine from the small intestinal loop in rats with diabetes (☆) or alcoholism ( ) or after pretreatment with no agent (control; ○), diethyl maleate (5 m*M* in administered solution; △), ethanol (10%; ▽), and salicylate (200 m*M*; □). The effect of cysteamine (see Fig. 1) on the disappearance rate of L-phenylalanine is represented by filled symbols. The dose of L-phenylalanine was 7.5 mg/loop. Each value represents the mean ± SD ($N = 4$). (a) $P < 0.05$ versus control; (b) $P < 0.1$ versus control.

within the first 7.5 min of incubation, and was not influenced by the presence of vanadate and Ca^{2+} in the medium (Table II).

Release of Ca^{2+} due to the presence of diethyl maleate into the Ca^{2+}-free medium is shown in Fig. 4. Since the Ca^{2+} concentration of the calibration curve (Fig. 4A), Ca^{2+} release into mucosal medium is represented by a change in the optical density of the medium. Ca^{2+} was released into the medium after 30 min of incubation when diethyl maleate was present, although neglectable Ca^{2+} release was observed in the absence of diethyl maleate. However, the Ca^{2+} release was small during the first 15 min of incubation with diethyl maleate, in spite of significant nonprotein thiol loss (Table II). Ca^{2+} release from the colonic mucosa after nonprotein thiol loss occurred more rapidly and to a greater extent in comparison to that from the ileal mucosa (Fig. 4B). Hence, the increase in CL_{pr} seems to correspond to Ca^{2+} release rather than to nonprotein thiol loss.

DISCUSSION

Living cell membranes show a greater barrier selectivity to the transport of hydrophilic compounds such as trypan blue (12,13), while membranes of dead cells allow the free permeation of trypan blue. This finding raises the question which common constituents of the living cells and/or cell membranes are essential in regulating the permeability

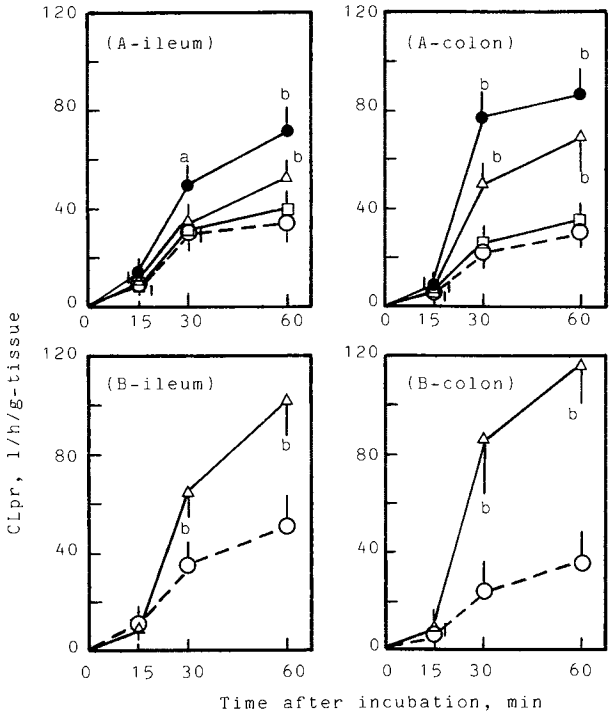

Fig. 3. Effect of diethyl maleate (1 m*M*; △), vanadate (2 m*M*; □), or both (●) on the phenol red clearance rate, CL_{pr}, as a function of incubation period, in the *in vitro* everted ileal and colonic sac experiments. (○) The result without any additive. Ca^{2+} medium was used for A and Ca^{2+}-free medium was used for B. Diethyl maleate was added only to the mucosal medium, but vanadate was added to both mucosal and serosal media. Each value represents the mean ± SD (N = 3 to 5). (a) $P < 0.1$ versus no additive; (b) $P < 0.05$ versus no additive.

to a wide range of solutes. In the intestinal mucosal membrane under normal conditions, nonprotein thiols appear to play an important role in preventing passive uptake of hydrophilic compounds. This hypothesis is supported by the following evidence. (i) A decrease in nonprotein thiols under disease conditions or after pretreatment with SH-modifying agents in the present study increased the absorption of cefoxitin, cefmetazole, and phenol red from the intestinal mucosa (Fig. 1). (ii) It has been reported that salicylate treatment allows the permeation of various hydrophilic compounds through rat rectal mucosa (13,14). (iii) Ethanol increases the absorption of hydrophilic compounds from the small intestine (15). (iv) Restoration of nonprotein thiols by cysteamine treatment suppressed the increased absorption of cefoxitin, cefmetazole, and phenol red that occurs under decreased nonprotein thiol concentrations (Fig. 1).

Further, in the *in vitro* everted intestinal sac study, a secondary event after nonprotein thiol loss may relate to the increase in phenol red transport for the following reasons. (i) The increase in CL_{pr} due to the presence of diethyl maleate in Ca^{2+}-free medium was greater than that in Ca^{2+} medium (Fig. 3). (ii) The increase in CL_{pr} occurred with a lag time after nonprotein thiol loss (Fig. 3 and Table II). (iii) Vanadate, which inhibits Ca^{2+} uptake into cells, accelerated the increase in CL_{pr} that was due to the presence of diethyl maleate (Fig. 3).

It has been reported that when nonprotein thiol loss occurs in hepatocytes, Ca^{2+} release from the extramitochondrial Ca^{2+} pool was also observed (16). From these findings and our present results, in which Ca^{2+} release from the intestinal mucosa occurred with a lag time after nonprotein thiol loss, nonprotein thiol loss may induce Ca^{2+} release from the intestinal mucosa, which is associated with in-

Table II. Concentrations of Nonprotein Thiol in the Ileal (I) and Colonic (C) Mucosae as a Function of Incubation Time, in the *in Vitro* Everted Intestinal Sac Study[a]

Condition and additive (intestine)	Nonprotein thiol (μmol/g protein) at incubation time			
	7.5 min	15 min	30 min	60 min
Ca^{2+} medium				
No additive				
I	13.4 ± 2.6	12.6 ± 1.4	12.7 ± 1.9	11.7 ± 1.1
C	10.5 ± 1.0	10.8 ± 1.9	10.0 ± 1.2	9.9 ± 1.0
DEM				
I	5.6 ± 1.4*	3.9 ± 0.9*	3.6 ± 1.4*	3.2 ± 1.1*
C	6.1 ± 1.9**	3.4 ± 0.6*	3.1 ± 0.4*	3.3 ± 0.8*
Vanadate				
I	12.6 ± 1.2	12.1 ± 1.4	12.2 ± 2.6	11.2 ± 1.3
C	11.1 ± 2.1	10.6 ± 1.9	10.2 ± 1.4	9.4 ± 1.6
Comb.				
I	6.4 ± 2.9**	3.6 ± 1.1*	3.5 ± 1.2*	3.1 ± 2.2*
C	4.0 ± 1.7*	3.9 ± 0.4*	3.1 ± 0.3*	3.4 ± 0.7*
Ca^{2+}-free medium				
No additive				
I	12.9 ± 1.2	12.1 ± 1.6	11.3 ± 2.1	11.4 ± 0.9
C	10.6 ± 1.9	10.1 ± 1.2	10.5 ± 0.7	9.7 ± 1.3
DEM				
I	4.9 ± 1.3*	3.4 ± 0.7*	3.4 ± 0.2*	3.5 ± 0.6*
C	5.9 ± 2.7**	3.2 ± 0.5*	3.6 ± 0.7*	3.1 ± 1.1*

[a] The incubation medium contained 1 m*M* diethyl maleate (DEM), 2 m*M* vanadate, or a combination (Comb.). Each value represents the mean ± SD (N = 3 or 4).

* $P < 0.05$ versus no additive.

** $P < 0.01$ versus no additive.

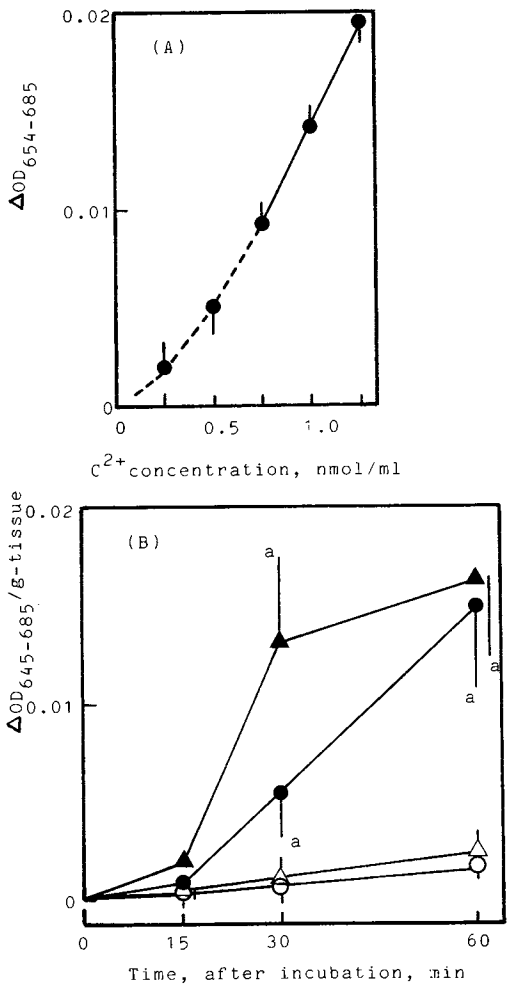

Fig. 4. (A) The calibration curve of Ca^{2+} concentrations. (B) Effect of diethyl maleate (1 m*M;* ● and ▲) on Ca^{2+} release from ileal (●) and colonic (▲) mucosa as a function of incubation period. (○) Ileum and (△) colon results when diethyl maleate was absent in the medium. Ca^{2+} release into the medium was represented by the change in optical density (OD) according to the method of Tone *et al.* (8) as described in Materials and Methods. ΔOD(654 − 685) in the figure was determined as follows: ΔOD(654 − 685) = {[OD(654) − OD(685)] for sample} − {[OD(654) − OD(685)] for deionized water}, where OD(654) and OD(685) represent the optical density of the sample at 654 and 685 nm, respectively.

creased mucosal membrane permeability to the passive transport of phenol red. Since Ca^{2+} release from the colonic mucosa occurred faster than that from the ileal mucosa (Fig. 4), it appears that Ca^{2+} homeostasis in the ileal mucosa after nonprotein thiol loss is more resistant than that in the colonic mucosa. This possible greater ileal resistance could explain the weak action of diethyl maleate in increasing CL_{pr} through the ileal sac in comparison with that through the colonic sac. It may similarly account for the weak effect of nonprotein thiol loss of the small intestine in increasing the absorption of cefoxitin, cefmetazole, and phenol red (Fig. 1) in the *in situ* intestinal loop study. However, it is not clear how Ca^{2+} release from the intestinal mucosa increases the intestinal mucosal permeability to the passive transport of hydrophilic compounds. Since it has been reported that strong chelating agents such as EDTA increase the passive transport of hydrophilic compounds in the intestine (17), some of the Ca^{2+} released from the intestinal mucosa may be a structural function in the mucosal membranes.

It is of interest that cysteamine, an exogenous nonprotein thiol, prevented the permeability of intestinal mucosal membrane to the passive transport of cefoxitin, cefmetazole, and phenol red with restoration of nonprotein thiol levels but did not restore the active transport of L-phenylalanine. This result indicates that exogenous nonprotein thiols such as cysteamine, as well as endogenous nonprotein thiols, can play a role in maintaining the cell membrane permeability, thereby preventing the passive transport of hydrophilic compounds.

Since it was reported that active transport of L-amino acids from the small intestinal lumen is mediated by glutathione, a major endogenous nonprotein thiol (18), the suppression of L-phenylalanine absorption under the disease conditions or after pretreatment with SH-modifying agents seems to be due to the decrease in endogenous nonprotein thiol concentrations in the mucosa. However, we did not further investigate the enzymatic system for the active transport of L-amino acids.

It has been reported that salicylate uncouples oxidative phosphorylation in mitochondria (19), and it is known that synthesis of glutathione requires ATP. Thus, a decrease in nonprotein thiol by salicylate may be due to partial impairment of glutathione synthesis. Further, increased amounts of the oxidized form of glutathione (GSSG) are effluxed by increasing activity of GSSG-ATPase (20). Thus, salicylate may inhibit GSSG-ATPase by decreasing the supply of ATP. The effect of salicylate in increasing intestinal permeability to the passive transport of hydrophilic compounds might occur by several mechanisms including the effect of salicylate in causing nonprotein thiol loss and a direct action of salicylate by binding to the mucosal membrane (21,22).

In conclusion, the increase in passive transport of hydrophilic compounds from intestines of rats with diabetes or alcoholism or after pretreatment with SH-modifying agents is associated with nonprotein thiol loss in the intestinal mucosa. The increase in passive transport further correlates with Ca^{2+} release from the intestinal mucosa, which occurs after nonprotein thiol loss. Cysteamine, an exogenous nonprotein thiol, can prevent the increased intestinal permeability to the passive transport of hydrophilic compounds subsequent to the loss of endogenous nonprotein thiols, while it did not restore the decreased active transport of L-phenylalanine.

REFERENCES

1. T. Nishihata, H. Takahata, and A. Kamada. *Pharm. Res.* 6:307–309 (1985).
2. N. S. Kosower. In *International Review of Cytology, Vol. 54,* Academic Press, New York, 1978, pp. 109–160.
3. S. B. Posalki and D. Rau. *Clin. Chim. Acta* 39:41–49 (1972).
4. G. L. Ellmann. *Arch. Biochem. Biophys.* 82:70–78 (1959).
5. S. Szabo, J. S. Trier, and P. W. Frankel. *Science* 214:200–202 (1981).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randal. *J. Biol. Chem.* 193:265–275 (1951).
7. W. H. Barr and S. Riegelman. *J. Pharm. Sci.* 59:70–78 (1970).
8. D. P. Tone, H. Thor, M. T. Smith, S. A. Jewell, and S. Orrenius. *J. Biol. Chem.* 258:6390–6393 (1983).

9. T. Nishihata, H. Takahagi, M. Yamamoto, H. Tomida, J. H. Rytting, and T. Higuchi. *J. Pharm. Sci.* 73:109–112 (1984).
10. T. Nishihata, K. Kamikawa, H. Takahata, and A. Kamada. *J. Pharmacobio-Dyn.* 7:143–150 (1984).
11. L. Caldwell, T. Nishihata, J. H. Rytting, and T. Higuchi. *J. Pharm. Pharmacol.* 34:520–522 (1982).
12. C. D. Klassen and N. H. Stacey. In G. Plaa and W. D. Hevit (eds.), *Toxicology of the Layer,* Raven Press, New York, 1982, pp. 147–179.
13. P. Sithigorngul, P. Burton, T. Nishihata, and L. Caldwell. *Life Sci.* 33:1025–1032 (1983).
14. T. Nishihata, J. H. Rytting, T. Higuchi, L. J. Caldwell, and S. J. Selk. *Int. J. Pharm.* 21:239–248 (1984).
15. W. L. Hayton. *J. Pharm. Sci.* 64:1450–1456 (1975).
16. S. A. Jewell, G. Bellomore, H. Thor, S. Orrenius, and M. T. Smith. *Science* 210:1257–1259 (1982).
17. T. Suzuka, T. Nishihata, M. Yamazaki, and A. Kamada. *Chem. Pharm. Bull.* 33:4600–4605 (1985).
18. A. Meister. *Science* 180:33–34 (1973).
19. T. M. Brody. *J. Pharmacol. Exp. Ther.* 117:39–51 (1956).
20. P. Nicotera, M. Moore, G. Bellomo, F. Mirabelli, and S. Orrenius. *J. Biol. Chem.* 260:1999–2002 (1985).
21. T. Nishihata and T. Higuchi. *Biochim. Biophys. Acta* 775:269–271 (1984).
22. H. Kajii, T. Horie, M. Hayashi, and S. Awazu. *Life Sci.* 37:523–530 (1985).