[Chem. Pharm. Bull. 36(5)1847—1856(1988)]

Intestinal Absorption of Drugs in Rats with Glycerol-Induced Acute Renal Failure

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(Received October 16, 1987)

The intestinal absorption of drugs was investigated in rats with glycerol-induced renal failure by an *in situ* loop method. Drugs examined were poorly-absorbable drugs (sulfanilic acid, procainamide ethobromide, cefazolin and sulfaguanidine), well-absorbable drugs (sulfisoxazole, quinine, salicylic acid and imipramine), actively-transported drugs (cefadroxil and cyclacillin) and water-soluble, high-molecular-weight compounds (polyethylene glycol (PEG) 1000, PEG 1500 and fluorescein isothiocyanate-conjugated dextran with a molecular weight of 4000). The absorption of all the low-molecular-weight drugs was significantly increased in the renal failure group, regardless of the absorption characteristics. The enhancement of membrane permeability was also observed by an *in vitro* cannulated everted sac method. The investigation of membrane permeability to high-molecular-weight compounds with PEG 1000 and 1500 showed that the enhancement of membrane permeability in the renal failure state was limited to molecules whose molecular weights were lower than about 1000. Furthermore, the enhancement of membrane permeability was seen in the brush border membrane vesicles prepared from the small intestine of rats with renal failure, although lipid fluidity, as assessed by steady-state fluorescence polarization techniques using 1,6-diphenyl-1,3,5-hexatriene as a probe was not changed in brush border membranes of the diseased rat. On the other hand, a reduction of thickness of the unstirred water layer adjacent to the membrane was observed. Examination by transmission electron microscopy revealed blebs at the tip of microvilli and the thickness of the glycocalyx was reduced. Possible mechanisms of the increase in drug absorption are discussed separately for poorly-absorbable and well-absorbable drugs.

Keywords——renal failure; drug absorption; poorly-absorbable drug; well-absorbable drug; brush border membrane; unstirred water layer; polyethylene glycol 1000; electron microscopy; rat small intestine

Since drug elimination is delayed during renal failure in general, it is possible that the increase in drug absorption induces a marked accumulation of the drug in the body, resulting in unexpected side-effects. Therefore, it is important to characterize the change in the intestinal absorption of drugs in this disease state. In the previous communication,[1] we reported the reduction of barrier function of the intestinal mucosa during acute renal failure, which was induced by an intramuscular injection of nephrotoxic substances, $HgCl_2$ or glycerol, or 5/6 nephrectomy.

In this study, the intestinal absorption of drugs was investigated in rats with glycerol-induced acute renal failure. The possible mechanisms are discussed.

Materials and Methods

Materials——Procainamide ethobromide (PAEB; Squibb Institute for Medical Research, Princeton, NJ,

U.S.A.), imipramine hydrochloride (Fujisawa Pharmaceutical Co., Osaka), cefazolin (Fujisawa Pharmaceutical Co.), cefadroxil (Bristol Meyers Co., Tokyo) and cyclacillin (Takeda Pharmaceutical Industries, Osaka) were used as supplied. Fluorescein isothiocyanate-conjugated dextran (FITC-D, molecular weight of 4000) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ^{14}C-Mannitol (NEN Research Products, Boston, MA, U.S.A.), ^{14}C-lauryl alcohol (ICN Pharmaceuticals, Irvine, CA, U.S.A.) and ^{3}H-dextran (Amersham International, Buckinghamshire, England) were used without further purification. Other reagents used in this study were of reagent grade.

Preparation of Rats with Renal Failure——Acute renal failure was induced in male Wistar rats weighing 180—240 g by an intramuscular injection of 50% glycerol (10 mg/kg).[2] Blood urea nitrogen (BUN) was monitored as an indicator of the extent of renal failure.

Procedure of Absorption Experiments——*In Situ* Experiments: Absorption experiments were carried out at 24 h after the glycerol injection. The absorption from the small intestine was examined by the *in situ* loop method.[3] Briefly, under pentobarbital anesthesia, the small intestine was exposed by a midline abdominal incision and a loop from the proximal end of the duodenum to the ileo-cecal junction was formed by cannulating both ends with silicone tubing. Care was taken to exclude major blood vessels from the ligatures. The bile duct was ligated in all the experiments. The contents of the lumen were washed out with saline warmed to 37 °C, then an isotonic aqueous solution of each drug was injected into the loop and each cannula was clamped with forceps. The organs were returned to the abdomen, and the incision was closed. The buffering salts of the isotonic solution were Na_2HPO_4–NaH_2PO_4 at pH 6.5. The volume of the solution injected was 5 ml. After the specified time, the solution in the segment was withdrawn and the lumen was washed with the same buffer solution. The solution and the washings were combined and the volume was adjusted to 50 ml. The amount of the drug absorbed was calculated as the difference in amount between the initial and final solutions.

The absorption from the large intestine (from the proximal end of the colon to the anus) was examined by an *in situ* loop method similar to that for the small intestine. The isotonic phosphate buffer solution (pH 6.5) was also used here. The volume of the solution injected was 2 ml.

The absorption from the stomach was examined by the *in situ* loop method of Schanker *et al.*[4] The isotonic solution (pH 1.1) was composed of 0.1 N HCl and NaCl. The volume of the solution injected was 4 ml.

In Vitro Experiments: The *in vitro* absorption kinetics were followed with the use of a cannulated everted sac method slightly modified from that described by Jorgensen *et al.*[5] A 5 cm portion of the mid-jejunum was used. Both ends of the everted sac were cannulated, and serosal samples were withdrawn from the lower cannula, while the contents were replenished by introducing fresh solution through the upper cannula. The buffer solution was the same one as used in the *in situ* absorption experiments. The volumes of mucosal and serosal solutions were 15 and 1 ml, respectively. The drug-containing mucosal solution was gassed with 95% O_2–5% CO_2 throughout the experiment. The initial drug concentration in the mucosal solution was 1 mM. The time course of the mucosal-to-serosal drug transport was examined by measuring the drug in serosal solutions.

Measurement of Unstirred Water Layer (UWL)——The UWL was estimated from the uptake rate of lauryl alcohol by the jejunal mucosa according to the method of Thomson.[6] A sheet of isolated rat jejunum was fixed to the chamber of Thomson. The mucosal and serosal solutions were Krebs-bicarbonate buffer solution (pH 7.4) and their volumes were 80 and 1 ml, respectively. After the preincubation for 5 min in the mucosal solution without lauryl alcohol, the mucosal solution was replaced by the buffer solution containing ^{14}C-lauryl alcohol and ^{3}H-dextran and the incubation was carried out for 6 min at 37 °C without or with stirring at a rate of 600 rpm. Lauryl alcohol taken up by the mucosa determined by measuring the radioactivity in the tissue cut off with a steel punch (5 mm diameter) and the alcohol content in the adhering external solution was corrected accordingly. The effective resistance of UWL ($d/S_w \cdot D$) was calculated from the rate of uptake without stirring by applying the following equation.

$$d/S_w \cdot D = C_1/J_d$$

where d and S_w are the thickness and the surface area of UWL, respectively, D is the diffusion coefficient of lauryl alcohol, C_1 is lauryl alcohol concentration in the mucosal solution, and J_d is the uptake rate of lauryl alcohol.

Estimation of Permeability of Brush Border Membrane——Brush border membrane vesicles were prepared from rat small-intestinal mucosa according to the method of Kessler *et al.*[7] The permeability of the brush border membrane was estimated by determining the release rate of ^{14}C-mannitol from the membrane vesicles. The vesicles were suspended in 300 mM mannitol–10 mM HEPES/Tris (pH 7.5) buffer (buffer A) containing ^{14}C-mannitol and preincubated for 60 min at 25 °C. Then, 20 μl of preloaded vesicles were diluted by the addition of 200 μl of buffer A and incubated at 25 °C. At selected times, 5 ml of ice-cold buffer A (stop solution) was added and the mixture was immediately transferred onto a cellulose nitrate membrane filter (0.45 μm pore size, Toyo Roshi Co., Tokyo). Then, the filter was rapidly rinsed with 5 ml of the ice-cold stop solution. After drying the filter, the radioactivity trapped on it was determined by liquid scintillation counting.

Estimation of Lipid Fluidity of Brush Border Membranes——Lipid fluidity of brush border membranes was estimated by the steady-state fluorescence polarization technique using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe as described by Schachter and Shinitzky.[8] Fluorescence polarization studies were performed with a Hitachi

MPF-4 spectrofluorometer (Hitachi, Tokyo) equipped with polarizers and thermoregulated cells. Fluorescence polarization was expressed as the fluorescence anisotropy. The microviscosity of the brush border membrane was calculated from the anisotropy of DPH.[9]

Blood Flow Measurements——The measurement of gastrointestinal blood flow was performed by means of a hydrogen clearance method (PHG 201, Unique Medical Co.).[10] Rats were anesthetized with pentobarbital. A platinum electrode was inserted into the intestinal wall. About 10% hydrogen gas was administered by inhalation for 3—5 min and the clearance constant was determined from the clearance curve plotted semilogarithmically.

Transmission Electron Microscopic Observation of Intestinal Epithelial Layer——Under pentobarbital anesthesia, the jejunum (about 5 cm below the ligament of Treitz) and the ileum (about 5 cm above the ileo-cecal junction) of rats were isolated. The tissue was fixed in 2% glutaraldehyde in cacodylate buffer for 1 h and post-fixed in 1% osmium tetroxide in Millonig buffer for 1 h. After dehydration in a graded ethanol series and embedding in Epon 812, the specimens were cut on a Reyhert Ultracut E (Reychert, Austria), stained with uranyl acetate and lead citrate for 3 min each, and examined in a JEM 100CX electron microscope (JEOL Ltd., Tokyo) at 80 kV.

Analytical Methods——Sulfanilic acid, PAEB, sulfaguanidine and sulfisoxazole were determined colorimetrically by the analytical procedures for sulfonamides.[11]

Quinine,[12] imipramine,[12] and salicylic acid[13] were determined spectrophotometrically as described previously.

FITC-D in the solution was determined fluorometrically with a Shimadzu RF-540 spectrofluorophotometer (excitation wavelength, 495 nm; emission wavelength, 514 nm). FITC-D in the intestinal tissue was determined as follows. The tissue was homogenized in distilled water and the homogenates were deproteinized with 10% trichloroacetic acid. The supernatant fraction was neutralized by the addition of NaOH solution and the fluorescence was determined as described above.

Cefazolin, cefadroxil and cyclacillin were determined by high performance liquid chromatography (HPLC). A high-pressure liquid chromatograph (LC-5A, Shimadzu,Kyoto) equipped with an ultraviolet detector (SPD-2A, Shimadzu) was used in a reversed-phase mode with an Inertsil ODS5 column (150 mm × 4.6 mm i.d., Gasukuro Kogyo Co., Tokyo). Mobile phases for the assay of cefazolin, cefadroxil and cyclacillin were methanol–20 mM phosphate buffer (pH 7.5) containing 5 mM tetra-*n*-butylammonium bromide (35 : 6), methanol–10 mM $NH_4H_2PO_4$ (1 : 9) and methanol–10 mM $NH_4H_2PO_4$ (25 : 75), respectively, and the flow rates were maintained at 1 ml/min. The wavelengths of the detector were 270, 262 and 210 nm for cefazolin, cefadroxil and cyclacillin, respectively. An aliquot of the sample solution was filtered through 0.45 μm pore size membrane filter (Japan Millipore Ltd., Tokyo) and an appropriate volume of the filtrate was injected into the liquid chromatograph. The drug concentration was calculated from the calibration line constructed on the basis of peak-area measurements.

Polyethyleneglycol (PEG) 1000 and PEG 1500 in aqueous solutions were determined by HPLC.[14] A high-pressure liquid chromatograph (LC-5A, Shimadzu) equipped with a refractive index detector (ERC-7510, Erma Optical Works Ltd, Tokyo) was used in a reversed-phase mode with a Shim-pack CLC-C8 (150 mm × 5 mm i.d.; Shimadzu). Mobile phases for the assay of PEG 1000 and PEG 1500 were methanol–water (46 : 54 and 50 : 50, respectively), and the flow rate was maintained at 0.9 ml/min. The sample solution was extracted with chloroform and an aliquot of the organic phase was evaporated to dryness at 40 °C. The residues of PEG 1000 and PEG 1500 were dissolved in each mobile phase. After filtration of the solution through a membrane filter (0.45 μm pore size; Japan Millipore Ltd.), an aliquot of the filtrate was injected into the high-pressure liquid chromatograph. The voltage output was integrated with a Chromatopack C-R1B integrator (Shimadzu). The molecular weight of each peak on the chromatogram was assigned by mass-spectroscopic measurement. BUN was determined by a diacetylmonoamine method.[15]

Statistical Analyses——Results were expressed as the mean ± standard error (S.E.). Statistical analyses were performed by using Student's *t* test.

Results

Intestinal Absorption of Drugs in Rats with Acute Renal Failure

Table I summarizes the absorption of drugs from the small intestine in normal rats and rats with acute renal failure, examined by an *in situ* loop technique. Model drugs in this table have a variety of absorption characteristics, *i.e.* polar poorly-absorbable drugs (PAEB, cefazolin, sulfanilic acid and sulfaguanidine), lipophilic well-absorbable drugs (sulfisoxazole, quinine and imipramine), a polar well-absorbable drug (salicylic acid), actively-transported drugs (cefadroxil[16] and cyclacillin[17]), and a polar macromolecule (FITC-D). As is evident from the table, the absorption of all the drugs except FITC-D was increased in the disease state, regardless of the absorption characteristics. In the case of cyclacillin, the higher the initial concentration was, the higher the rate of increase became. On the other hand, the

TABLE I. Intestinal Absorption of Drugs in Rats with Glycerol-Induced Renal Failure

Drug	Drug concentration (mM)	Time (min)	Absorption (%)	
			Normal	Renal failure
PAEB	1.0	60	11.1±1.1 (4)	19.3±2.3 (8)[a]
Cefazolin	1.0	60	14.3±1.6 (6)	24.5±2.1 (4)[b]
Sulfanilic acid	1.0	60	18.9±1.5 (6)	25.2±1.5 (3)[a]
Sulfaguanidine	1.0	60	22.7±1.3 (4)	34.2±1.9 (3)[b]
Sulfisoxazole	1.0	10	34.3±2.9 (5)	51.5±1.8 (5)[b]
Quinine	1.0	10	39.7±1.4 (4)	53.2±2.8 (4)[b]
Salicylic acid	1.0	5	47.2±2.6 (4)	60.9±0.5 (3)[b]
Imipramine	1.0	5	47.3±1.6 (4)	52.9±1.6 (5)[a]
Cefadroxil	1.0	10	53.3±0.8 (4)	67.5±1.7 (4)[c]
Cyclacillin	0.1	5	53.8±1.5 (4)	63.4±1.3 (4)[b]
	1.0	5	48.2±2.1 (7)	64.9±4.4 (3)[b]
	10.0	5	22.3±1.3 (4)	35.5±2.5 (3)[b]
FITC-D[d]	0.1 (mg/ml)	60	4.0±0.7 (4)	5.5±1.0 (7)

Mean BUN values were 14.8±0.5 and 113.8±3.5 mg/dl in normal rats and rats with renal failure, respectively. Results are expressed as the mean±S.E. with the number of experiments in parentheses. *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$, compared with each normal value. *d*) Molecular weight of 4000.

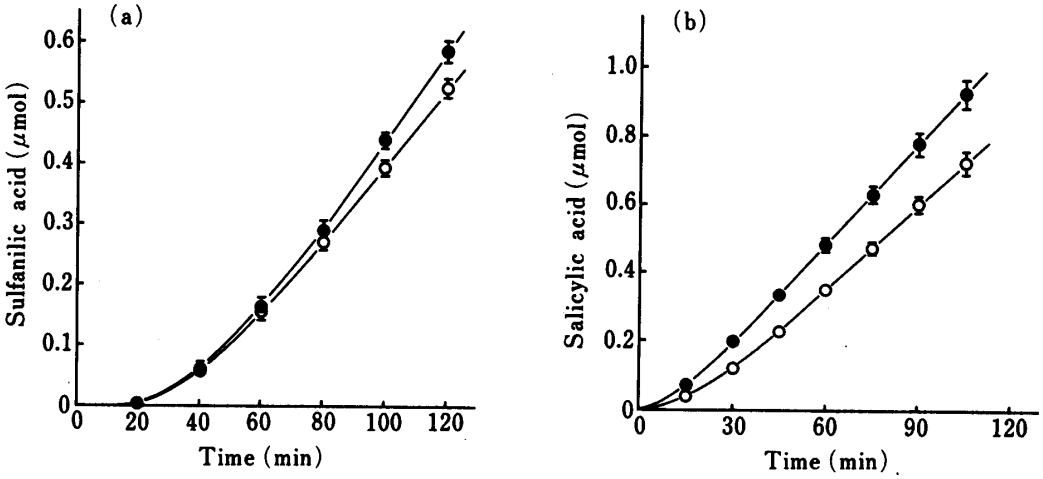

Fig. 1. Cumulative Mucosal-to-Serosal Transfer of Sulfanilic Acid (a) and Salicylic Acid (b) through Cannulated Everted Sacs of Small Intestine Isolated from Rats with Renal Failure

○, normal (mean BUN: 11.3±1.1 mg/dl); ●, renal failure (mean BUN: 133.8±9.7 mg/dl). Results are expressed as the mean±S.E. of 3—5 experiments.

absorption of FITC-D (molecular weight, 4000) was not increased.

The increased drug absorption in acute renal failure was also observed in the isolated intestine. Figure 1 shows the results of mucosal-to-serosal transfer of sulfanilic acid (a) and salicylic acid (b) in the jejunum isolated from normal rats and rats with renal failure. The increase in the permeability to sulfanilic acid seems to be small but the difference between the two slopes is statistically significant. Thus, it is evident that the permeability of the small-intestinal membrane is increased in the state of acute renal failure.

Measurement of Intestinal Permeability to Different-Sized PEGs

Since the absorption of FITC-D with a molecular weight of 4000 was not changed in acute renal failure as described above, the intestinal permeability in this disease state was investigated using different-sized PEGs. PEG 1000 and PEG 1500 are mixtures of HO–(CH_2–

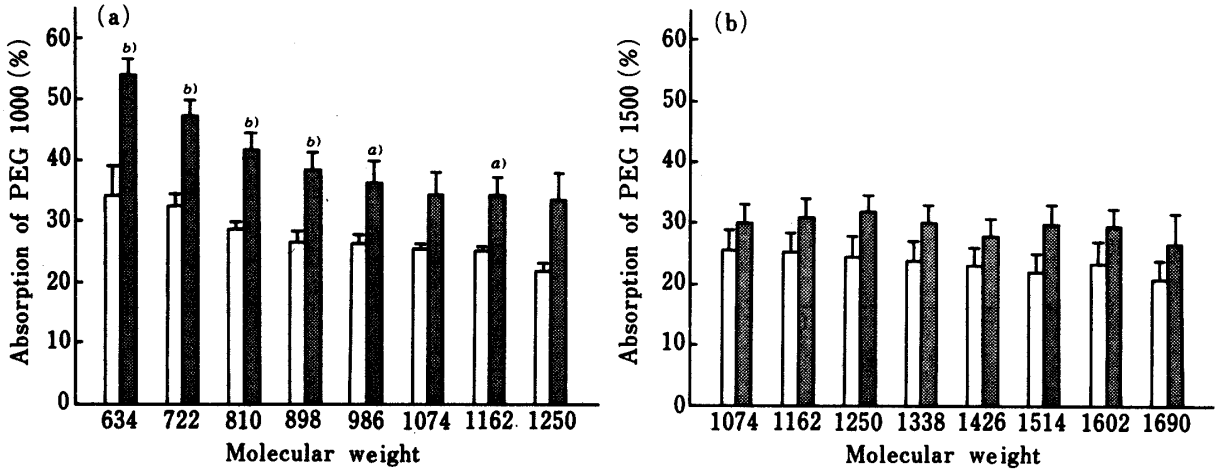

Fig. 2. Intestinal Absorption of Different-Sized PEG 1000 (a) and 1500 (b) in Rats with Renal Failure

Mean BUN values were 14.5±1.2 and 128.1±13.5 mg/dl in normal rats and rats with renal failure, respectively. The absorption was examined for 1 h by an *in situ* loop method. The initial concentration of PEGs was 10 mg/ml. The results are expressed as the mean±S.E. of 4—5 experiments. Results from normal rats and rats with renal failure are shown as open and dotted columns, respectively. *a*) $p<0.05$; *b*) $p<0.01$, compared with each normal value.

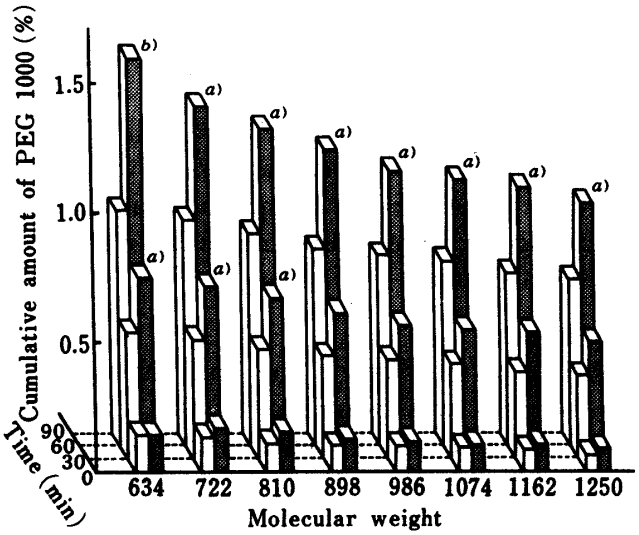

Fig. 3. Cumulative Mucosal-to-Serosal Transfer of PEG 1000 through Cannulated Everted Sacs of Small Intestine Isolated from Rats with Renal Failure

Mean BUN values were 15.8±1.0 and 148.5±17.5 mg/dl in normal rats and rats with renal failure, respectively. The initial concentration of PEG 1000 in the mucosal solution was 20 mg/ml. The results of 4—5 experiments from normal rats and rats with renal failure were shown as open and dotted columns, respectively. *a*) $p<0.05$; *b*) $p<0.01$, compared with each normal value.

$CH_2O)_nH$ with different n, and their average molecular weights are about 1000 and 1500, respectively. The absorption of these different-sized PEGs could be examined simultaneously by HPLC analysis.

Figure 2 shows the effect of acute renal failure on the intestinal absorption of PEG 1000 (a) and PEG 1500 (b), examined by an *in situ* loop method. Smaller-sized PEGs tended to be absorbed better in normal rat intestine. In the disease state, the absorption of PEG with a molecular weight of less than 1000 was increased, while the absorption of the higher-molecular-weight parts of PEG 1000 and PEG 1500 was unaltered.

The membrane permeability to PEG 1000 was investigated in intestines isolated from normal and diseased rats. As shown in Fig. 3, the permeability increase was observed for all fractions of PEG 1000, but it is evident that the permeability to smaller molecules increased markedly. The results of these *in situ* and *in vitro* experiments indicate that the increased membrane permeability in the renal failure state was limited to molecules whose molecular weights were lower than about 1000.

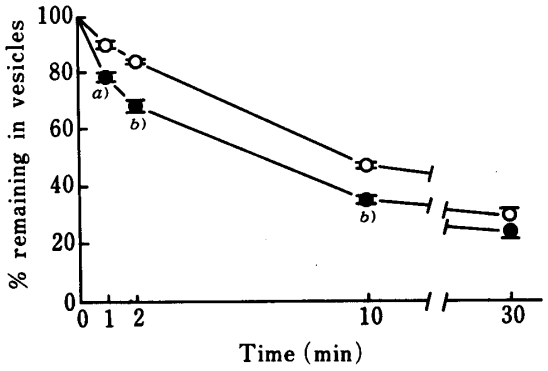

Fig. 4. Release of ^{14}C-Mannitol from Preloaded Brush Border Membrane Vesicles

○, normal (mean BUN: 17.1±1.1 mg/dl); ●, renal failure (mean BUN: 131.1±1.2 mg/dl). Results are expressed as the mean±S.E. of three experiments. *a*) $p<0.05$; *b*) $p<0.001$, compared with each normal value.

TABLE II. Fluorescence Polarization of DPH in Small-Intestinal Brush Border Membranes of Normal Rats and Rats with Renal Failure

Rat	Fluorescence anisotropy	Microviscosity (P)
Normal (4)	0.238±0.002	4.58±0.10
Renal failure (3)	0.243±0.002	4.90±0.13

Mean BUN values were 18.0±2.1 and 168.1±3.0 mg/dl in normal rats and rats with renal failure, respectively. All values are for 37 °C and are expressed as the mean±S.E. Numbers of animals are shown in parentheses.

Measurement of Permeability to Brush Border Membranes

In order to clarify the change in the permeability of intestinal brush border membrane in the disease state, the release rate of mannitol from brush border membrane vesicles was examined. As shown in Fig. 4, the release rate of the marker compound was markedly increased in rats with acute renal failure, indicating the increased permeability of the apical plasma membrane of the epithelial cell in the disease state.

Change in Lipid Fluidity of Brush Border Membranes

Since one of the factors affecting membrane permeability is the lipid fluidity of the membrane, the fluidity of brush border membranes was examined by the fluorescence polarization technique using DPH as a probe. The results are summarized in Table II. Lipid fluidity of the brush border membrane was not significantly different between normal and renal failure states.

Change in UWL

There are two barriers for drug permeation across the mucosal membrane; one is the lipoid membrane of the epithelial cell and the other is UWL adjacent to the membrane. It is well known that UWL acts as a major barrier for the transport of highly-lipophilic compounds.[18] In order to clarify the change in UWL, the rate of lauryl alcohol uptake by jejunal mucosa was examined with or without stirring of the mucosal solution. As shown in Table III, the uptake was markedly increased by the stirring, indicating that the transfer across UWL is the rate-limiting step for lauryl alcohol uptake. The uptake rate without stirring was significantly higher in the disease state, while the uptake rate under stirring (600 rpm) decreased. The calculated effective resistance of UWL was significantly decreased in the disease state; 243±28 and 132±18 min/ml/100 mg, for the intestines from normal rats and rats with renal failure, respectively.

Measurement of Mesenteric Blood Flow Rate

The rate of mesenteric blood flow was examined in normal rats and rats with acute renal

TABLE III. Effect of Stirring on Mucosal Uptake of Lauryl Alcohol in Small Intestines of Normal Rats and Rats with Renal Failure

Rate of stirring (rpm)	Rate of uptake (nmol/100 mg/min)	
	Normal	Renal failure
0	0.430±0.052 (4)	0.813±0.132 (4)[a]
600	41.2 ±2.5 (4)	21.8 ±2.8 (5)[b]

Mean BUN values were 17.9±0.9 and 89.4±13.2 mg/dl in normal rats and rats with renal failure, respectively. Results are expressed as the mean±S.E. with the number of experiments in parentheses. *a*) $p<0.05$; *b*) $p<0.01$, compared with each normal value.

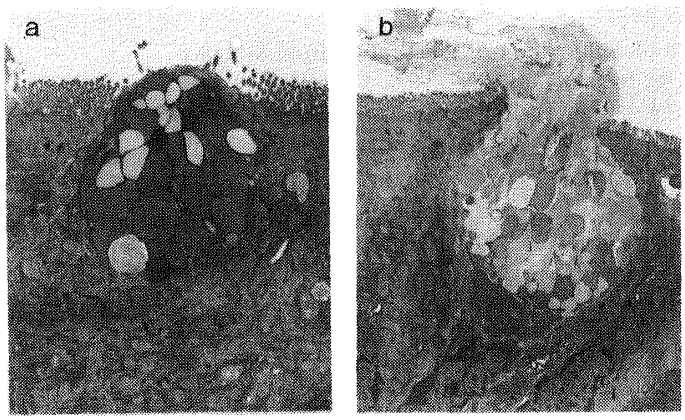

Fig. 5. Transmission Electron Micrograph of Goblet Cells in Small Intestines of Normal Rats (a) and Rats with Renal Failure (b) (×6600)

TABLE IV. Effect of Renal Failure on Secretion of Goblet Cell Mucin in Rat Small Intestine

	No. of secreting cells	Total No. of counted cells	% of secreting cells
Jejunum			
Normal	7	138	5.1
Renal failure	22	110	20.0
		$\chi^2=11.80$	$p<0.001$
Ileum			
Normal	13	160	8.1
Renal failure	35	171	20.5
		$\chi^2=8.88$	$p<0.01$

Goblet cells of semithin sections were counted by photomicroscopy after staining with toluidine blue.

failure by the hydrogen clearance method. The blood flow was not changed in the disease state; 226±17 and 217±11 ml/min/100 g in the normal and diseased rats, respectively.

Morphological Changes in Intestinal Mucosa

Figure 5 shows typical electron micrographs of a goblet cell from the intestine of normal rats and rats with renal failure. A number of violently secreting goblet cells are observed in the disease state. The accelerated secretion was quantitatively assessed by examining intestinal goblet cells in semithin sections stained with toluidine blue. As shown in Table IV, secreting cells are evidently increased in the disease state, suggesting catarrh. Figure 6 shows electron micrographs of the brush border membranes. Blebs were found at the tip of microvilli, and the thickness of glycocalyx was relatively reduced in the disease state. On the other hand, no

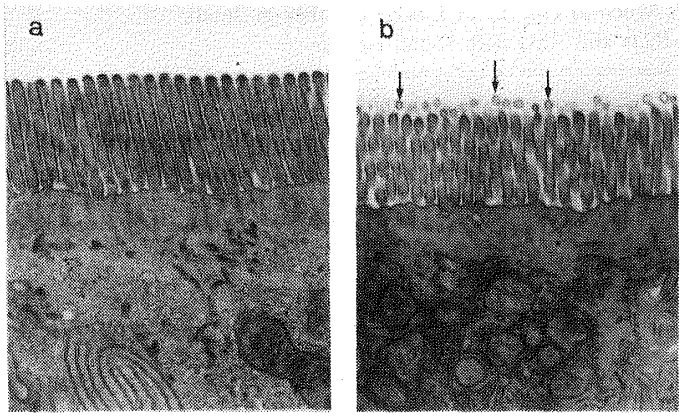

Fig. 6. Transmission Electron Micrograph of Brush Border Membranes in Small-Intestinal Epithelial Cells of Normal Rats (a) and Rats with Renal Failure (b) (× 16600)
Arrows indicate blebs at the tip of microvilli.

TABLE V. Effect of Renal Failure on Sulfanilic Acid Absorption from Stomach and Large Intestine in Rats

Site	% absorbed in 1 h	
	Normal	Renal failure
Stomach	1.1±0.4 (4)	2.9±1.3 (6)
Large intestine	23.0±2.9 (7)	30.0±1.8 (7)

Mean BUN values were 13.6±0.7 and 117.0±7.5 mg/dl in normal rats and rats with renal failure, respectively. Results are expressed as the mean±S.E. with the number of experiments in parentheses.

change was observed in the tight junction area (not shown).

Drug Absorption in Stomach and Large Intestine

In order to investigate whether the enhanced permeability in the renal failure state is also observed in the stomach and the large intestine, absorption of sulfanilic acid was examined in these organs of the diseased rat by *in situ* loop methods. The results are shown in Table V. The absorption in both organs tended to increase in the disease state, but the increases were not statistically significant.

Discussion

It has been shown that not only reduction of renal excretion but also changes in hepatic metabolism and plasma protein binding of drugs occur in renal failure states.[19,20] Our preliminary report[1] and Table I in this report showed that drug absorption from the small intestine was increased in rats with experimental acute renal failure. It is important to characterize the change in the intestinal absorption of drugs in the renal failure state, since reduction of renal excretion might magnify the drug accumulation in the body. The mechanism of the increase in drug absorption will be discussed separately for poorly-absorbable and well-absorbable drugs.

Increased Absorption of Poorly-Absorbable Drugs

As is evident from Table I, the intestinal absorption of various poorly-absorbable, low-molecular-weight drugs was significantly increased in the renal failure state, suggesting a reduction of intestinal barriers to drug absorption in general. Experiments in the everted intestine showed an enhancement of membrane permeability. As shown in Fig. 5 and Table IV, secreting goblet cells are increased in the intestine of rats with renal failure. The relationship between the increase in the secretion and the enhancement of intestinal

permeability is not clear. However, it is generally known that intestinal catarrh is accompanied with increased secretion. Thus, it is suggested that the intestinal condition is abnormal in the renal failure state. Further investigation of membrane permeability using PEG 1000 and 1500 showed that the enhancement of membrane permeability in the disease state was limited to molecules whose molecular weights were lower than about 1000 (Figs. 2 and 3).

The results of voltage-clamp experiments[21] using sulfanilic acid as a model drug suggest that the enhancement of intestinal permeability in the renal failure state was mainly due to increased permeability of the transcellular route. This agrees well with the enhanced permeability of brush border membranes to mannitol (Fig. 4). The observation that the absorption enhancement is limited to low-molecular-weight drugs is also consistent with the change in the transcellular permeability. Although lipid fluidity in the hydrophobic regions of the brush border membrane did not change in the disease state (Table II), blebs seen at the tip of the microvilli on the electron micrographs (Fig. 6) indicate disturbances of the membrane architecture and might have a close connection with the enhanced permeability of the brush border membrane. Membrane fluidity studies are in progress by using fluidity probes localized in different parts of the membrane.

As shown in Table V, the enhancement of sulfanilic acid absorption was observed neither in the stomach nor in the large intestine of rats with renal failure. McDermott *et al.* reported that cell divisions of epithelial cells in the esophagus[22] and the small intestine[23] were inhibited in the renal failure state. On the hypothesis that the inhibition of the cell division of epithelial cells in the alimentary tract results in the reduction of the barrier function and the enhanced permeability to drugs, the effect would be expected to be more prominent in an organ with epithelial cells having short life-cycles, such as the small intestine, in comparison with the stomach and the large intestine.[24] This explains well the results described above.

Increased Absorption of Well-Absorbable Drugs

Table I shows that renal failure causes an increase in the intestinal absorption of well-absorbable drugs too. Well-absorbable drugs are mostly lipophilic. A major barrier for the permeation of such drugs might not be the lipoid membrane of epithelial cells but UWL adjacent to the membrane.[18] The experimental result that the rate of uptake of lauryl alcohol by the intestinal mucosa was increased in renal failure rats suggests a reduction of the UWL thickness. The increased absorption of lipophilic well-absorbable drugs in the disease state can be explained by this change in UWL. The reduction of the thickness of glycocalyx observed in electron micrographs of the brush border membrane (Fig. 6) agrees well with this assumption.

Three polar well-absorbable drugs, salicylic acid, cefadroxil and cyclacillin, were examined (Table I). The precise mechanism of salicylic acid absorption remains unsolved, but the enhanced permeability of the intestinal membrane to this drug could contribute to the increased absorption in the disease state (Fig. 1b). Both cefadroxil[16] and cyclacillin[17] can be absorbed from rat small intestine by an active transport mechanism. Electrophysiological investigation of the intestinal mucosal membrane of rats with renal failure showed no significant change in the short-circuit current, suggesting that active transport mechanisms of the epithelial cell are not altered in the disease state.[21] Wilson and Dietschy reported that a reduction of the UWL thickness results in low Michaelis constants, K_m, for active transport processes.[25] Since our data on cyclacillin absorption were obtained at only three concentrations, K_m values for the cyclacillin transport system cannot be estimated exactly here. Thus, the increase in the absorption of these drugs might be due to the enhancement of the passive transport part. The fact that the increase in the absorption of cyclacillin was almost uniform, around 10%, regardless of the initial concentration, supports this hypothesis.

Another factor which influences the absorption rate of well-absorbable drugs is the

mesenteric blood flow.[26] However, measurement of the mesenteric blood flow by means of a hydrogen clearance method revealed no difference between normal and renal failure states. Therefore, the blood flow factor should not contribute to the increase in the absorption of well-absorbable drugs in the renal failure group.

In conclusion, the intestinal absorption of passively-transported, low-molecular-weight drugs is increased in the renal failure state due to both an enhancement of membrane permeability, probably in the region of the brush border membrane, and a reduction of the thickness of UWL adjacent to the membrane.

Acknowledgement We should like to thank Drs. H. Sezaki and A. Yamamoto, Faculty of Pharmaceutical Sciences, Kyoto University, for their many helpful suggestions concerning blood flow measurements.

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