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Effect of Some Cationic Drugs on the Intestinal Absorption of Pralidoxime Iodide[1]

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The purpose of this study was to clarify the competition and the mechanism of intestinal absorption of various cationic drugs by *in situ* and *in vitro* techniques. As model drugs, pralidoxime iodide (2-PAM) and several amine drugs were employed. Although the absorption of 2-PAM and 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride (A.C.D.B.) became saturated when drug concentration increased, they might not be absorbed actively. Nine amine drugs including A.C.D.B. competitively inhibited the absorption of 2-PAM. Furthermore, the absorption of A.C.D.B. was similarly inhibited by one of the amine drugs. No physicochemical interaction as well as direct action on the membrane permeability were observed. Since A.C.D.B. did not affect the absorption of quinine and L-tryptophan, its effect is thought to be brought on the specified drugs. Inhibitory action of the amine drugs on the accumulation of 2-PAM to the gut wall and the uptake to the epithelial cells were operative. Since pharmacologic action of amine drugs is so varied and the drugs with opposite pharmacologic action inhibit 2-PAM absorption as well, it is supposed that the molecular structures which amine drugs have in common (phenylalkylamine) may relate to the inhibitory effect. Other cationic drugs having no such group did not affect the absorption of 2-PAM at all.

Quaternary ammonium compounds are poorly and irregularly absorbed from the gastrointestinal tract. These compounds are completely ionized and their transfer across the lipid membrane can not be explained on the basis of the classical theory of passive diffusion of the unionized species of a drug molecule.

During the last decade, Levine, *et al.* performed extensive studies of the intestinal absorption of benzomethamine, an anticholinergic drug.[3] Despite the fact that this quaternary ammonium compound could be detected in the urine after oral administration almost as fast as intravenous administration, the total amount recovered in the urine within four hours represented below 8% of the total dose, in sharp contrast to 18—50% recovery after intravenous injection. Evidence has been obtained which indicates that this poor absorption on oral administration is due to the cationic nature which promotes the formation of a non-absorbable complex with intestinal mucin.[4] The detailed studies of the absorption kinetics of benzomethamine revealed that more than a process of passive diffusion was involved.[5] They assumed that this quaternary drug is transferred across the gut wall as an neutral complex by virtue of combination with an endogeneous anion. Since the phosphatido-peptide fraction isolated from intestinal tissue enhanced the absorption of benzomethamine, it was supposed that this fraction might play an important role in the transfer process.[6]

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Another interesting report is about the mechanism of the intestinal absorption of phenol red,[7] which is an anionic compound and completely ionized at the physiological pH range. Though it is mainly absorbed by simple diffusion, in lower concentration range it is absorbed by an active transport mechanism of low capacity.

These examples may be extraordinary, but commonly, ionized drug molecules of poor lipid solubility are supposed to be absorbed by fairly analogous mechanisms. Currently, several hypotheses are offered about the absorption mechanism of ionized drugs,[8–10] and these drugs are supposed to be capable of penetrating the biological membrane alike nonionized drugs.[11] This may be due to the fact that ionized drug molecules have affinity to some constituents of the membrane,[12,13] however, the precise mechanism of the intestinal absorption of highly ionized drugs is still uncertain.

The purpose of this report is to investigate the mechanism of intestinal absorption of pralidoxime iodide (2-PAM) and several cationic drugs. On the mechanism of the intestinal absorption of mono-quaternary pyridinium aldoxime salts including 2-PAM, Levine and Steinberg suggested that they were unique in their rapid and near-complete absorption from the small intestine.[14] In 1969, Crone and Keen suggested that the transfer of pyridinium aldoximes across the wall of jejunal sacs of rats could be explained by passive diffusion through aqueous pore.[15] Kakemi, *et al.* revealed that the absorption of 2-PAM and its derivatives from the *in situ* rat intestine became saturated when their concentration increased and one derivative inhibited the absorption of the other.[16] On the other hand, a number of cationic drugs have a common structure in their molecules, namely aromatic and dialkylamine groups, and these groups participate in the surface and biological activity. Previous report from this laboratory has indicated that their intestinal absorption approaches a constant value when their concentration increases and their absorption is inhibited mutually.[17] Since these compounds also inhibit the intestinal absorption of 2-PAM, we have discussed about their interrelations.

Experimental

Materials——2-Allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide hydrochloride (A.C.D.B.) was a gift from Fujisawa Pharmaceutical Co., Ltd. 2-PAM and other drugs were of commercial quality. All other materials used were of reagent grade.

Analytical Methods——All spectrophotometric analyses were made with a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer and a Hitachi Model 101 spectrophotometer. Estimation of drugs in the gastrointestinal perfusate was as follows:

1) 2-PAM: One half ml of sample solution was placed in a 12 ml glass-stoppered centrifuge tube and 4 or 5 ml of 0.1 N hydrochloric acid and 5 ml of isoamyl alcohol saturated with distilled water were added. After the tube had been shaken vigorously for 30 min and centrifuged, 3 ml aliquots of aqueous phase was diluted adequately with 0.2 N sodium hydroxide and the absorbance was determined at 336 nm against a blank solution.

2) A.C.D.B.: A few ml of sample solution was placed in a 12 ml glass-stoppered centrifuge tube and 2 ml of 0.2 N sodium hydroxide and 5 ml of chloroform were added. After the tube had been shaken for

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15 min and centrifuged, the aqueous phase was removed. Three ml of organic phase was diluted with chloroform and the absorbance was measured at 288 nm against a blank solution.

3) Phenol red: One half ml of sample solution centrifuged previously was diluted with 0.2 N sodium hydroxide and the absorbance was measured at 557 nm.

4) L-Tryptophan: L-Tryptophan was determined by the procedure A of Spies and Chambers.[18]

5) Quinine: Quinine was determined as previously described.[19]

For the quantitative determination of 2-PAM in the intestinal tissue homogenate preparations and serosal fluid of *in vitro* transfer experiments, a few ml of sample solution was added to 1 ml of 20% trichloroacetic acid and shaken vigorously. For 2-PAM in isolated intestinal epithelial cells, cell pellet was homogenized in 5% trichloroacetic acid. After centrifugation, 2-PAM in the supernatant was estimated according to the method for the gastrointestinal perfusate. These estimations were calibrated using standard tissue preparations.

Procedure of *in Situ* Absorption Experiments——Nonfasted male rats of the Wistar strain, weighing from 140 to 170 g, were used throughout. The degree of intestinal absorption was determined using either the perfusion method or single-loop method. The animals were anesthetized with sodium pentobarbital.

1) Perfusion Method: The procedure previously reported was employed.[20] The perfusion rate was 8 ml per min and the volume of perfusate was 40 ml. After 10 min lag period, samples were withdrawn at 0 and 60 min for the estimation of drug and volume change indicator (phenol red) respectively. After 60 min perfusion, 97.4±1.4% of phenol red was recovered from the perfusate. Medium of the perfusate was isotonic sodium chloride having pH 6.5, unless otherwise mentioned.

2) Single-Loop Method: The proximal ligature was placed about 5 cm distal to the ligament of Treitz. The loop was about 10 cm long, drained by 5 mesenteric blood vessels. One half ml of drug solution was injected into the loop after washing out the lumen contents. After the appropriate time, the loop was removed and the quantity of 2-PAM in the lumen and tissue was estimated.

Procedure of *in Vitro* Everted Sac Method——Two 8 cm segments per rat were cut off from the jejunal site, everted, and tied to glass cannula. The technique was the modification of that of Crane and Wilson.[21] The everted sacs contained initially 0.8 ml standard Ringer solution[22] and were incubated in 8.0 ml standard Ringer solution containing 2-PAM (or 2-PAM with A.C.D.B.) for 60 min at 37° under aerobic conditions. At the end of the incubation the sacs were quickly rinsed, excess fluid removed on tissue paper and the serosal fluid volume determined by weighing. The amount of 2-PAM transferred to the serosal side and taken up to the gut wall were separately analyzed. The ratio of concentration of 2-PAM between serosal and mucosal fluid was determined as above mentioned method.

Experiments of 2-PAM Uptake by the Isolated Intestinal Epithelial Cells——Isolated intestinal epithelial cells were prepared by the method of Reiser and Christiansen.[23] About 300 mg (wet weight) of the isolated cells was added to 5 ml of Krebs-Ringer Tris buffer (pH 7.4) containing 118 mM NaCl, 25 mM Tris-HCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 2 mM 2-PAM (or 2 mM 2-PAM and 4 mM A.C.D.B.). After 15 min incubation at 37°, the reaction was terminated by pouring the contents of the incubation mixture into a centrifuge tube in an ice bath and centrifuging the cells at $200 \times g$ for 5 min in the cold. The cells were then washed and centrifuged a few times with 5 ml cold Krebs-Ringer Tris buffer. Final cell pellet was homogenized in 5% trichloroacetic acid and 2-PAM contained was determined. The uptake of 2-PAM by the cells was expressed as nmoles/mg cell protein. Since the final uptake after 5 washings with ice cold Krebs-Ringer Tris buffer was not significantly different from the uptake after 3 washings, the cell pellet was washed 4 times. The cell viability was examined microscopically after a 2% solution of trypan blue was added to an aliquot of the diluted cell suspension.[24] The initial (before incubation) and the final (after incubation and washings) cell viability were approximately 80% and 50% each. After a 15 min incubation at 37° and washings, shapes of cells became round. Smears of the pellet being stained with Giemsa's Stain, lymphocytes seemed to be contained considerably (about 10%).

Determination of Cell Protein——Cells were dissolved in 1 N sodium hydroxide and cell protein determined by the colorimetric method of Lowry, *et al.*[25] Bovine serum albumin was used as the reference standard.

Microscopic Examination of the Rat Small Intestine——Segments of the intestine removed epithelial cells were fixed in 9% formalin, embedded in paraffin and stained with haematoxylin and eosin.

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Results and Discussion

The Disappearance of Cationic Model Drugs from the Rat Small Intestine

The disappearance of 2-PAM and A.C.D.B. from the rat small intestine was investigated by the *in situ* perfusion method. Since the value of pK_a for oxime group of 2-PAM is 7.82,[26] 2-PAM exists largely in the forms of a monovalent cation at the pH of absorption experiments. Partition coefficients obtained in pH 6.5 phosphate buffer-chloroform system was approximately 0.02 and this value coincided with the previous report.[27] From these results, it was suggested 2-PAM had poor lipid solubility. Contrary to the physicochemical properties, it was comfirmed that the rates of disappearance were not so slow and proportionality was not observed between the initial drug concentration and the percentage of disappearance. (Fig. 1).

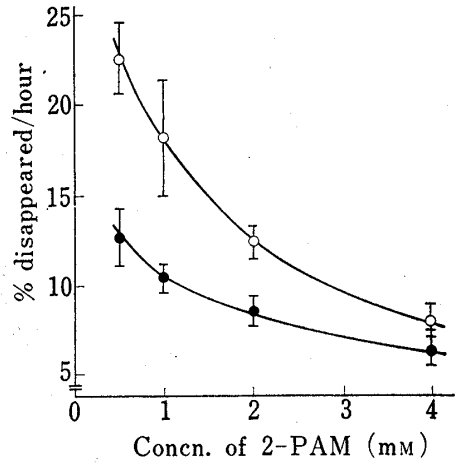

Fig. 1. Effect of A.C.D.B. on the Disappearance of 2-PAM from the Rat Small Intestine

Concentration of A.C.D.B.: 1 mM
key: ○ 2-PAM alone
● 2-PAM with A.C.D.B.
Each point represents the mean value of at least five experiments.
Vertical bars indicate s.d.

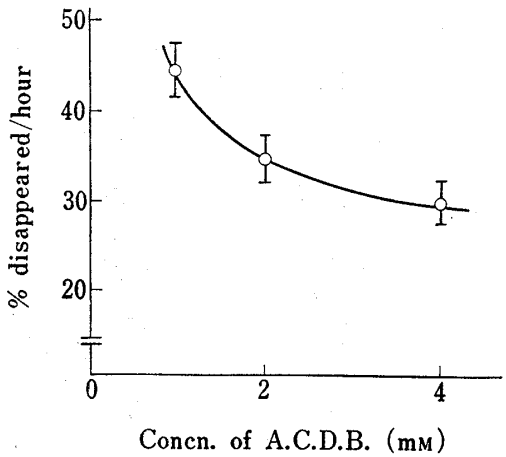

Fig. 2. Disappearance of A.C.D.B. from the Rat Small Intestine at Various Initial Concentrations

Each point represents the mean value of at least five experiments.
Vertical bars indicate s.d.

A.C.D.B. is a tertiary amine compound, having pK_a 7.9.[28] As shown in Fig. 2, when drug concentration increased, the disappearance of A.C.D.B. decreased and approached a constant value.

These results suggest that 2-PAM and A.C.D.B. would be absorbed by more than a process of passive diffusion.

Effect of Amine Drugs on the Disappearance of 2-PAM

As shown in Table I, the disappearance of 2-PAM was inhibited by nine amine drugs. Pharmacologic action of these amine drugs is so varied. For instance, diphenhydramine, chlorpheniramine, and carbinoxamine are classified into antihistaminics, dicyclomine into anticholinergic drugs, β-phenylethylamine into adrenergic drugs, and tyramine into adrenergic drugs or histamine releaser. It is interesting to note that the drugs with opposite pharmacologic action inhibit 2-PAM absorption as well.

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Table I. Effect of Various Amine Drugs on the Disappearance of 2-PAM from the Rat Small Intestine

Drug	Concn. (mM)	% disappeared per hour	% of the control	Water transfer ratio
Control	—	18.1±3.3	—	0.926±0.023
A.C.D.B. Blood[b]	*a*)	18.8±1.0	103.9	0.924±0.023
Lumen[c]	4.0	2.9±2.5	16.0	0.947±0.032
	pretreat[d]	21.0±2.4	116.0	0.894±0.035
Diphenhydramine	4.0	4.2±0.6	23.2	0.966±0.017
Carbinoxamine	4.0	2.0±1.5	11.0	0.994±0.020
Chlorpheniramine	4.0	6.8±2.3	37.6	0.961±0.029
Dicyclomine	2.0	4.4±1.5	24.3	0.997±0.018
Metoclopramide	0.5	14.0±2.6	77.3	1.000±0.028
Imipramine	1.0	9.0±3.7	49.7	0.906±0.029
β-Phenylethylamine	4.0	13.7±1.0	75.7	1.018±0.020
Tyramine	1.0	14.9±1.9	82.3	0.959±0.042

concentration of 2-PAM: 1 mM
a) 0.02 mmoles/rat
b) Immediately after 0.02 mmoles A.C.D.B. was administered intravenously, the disappearance of 2-PAM from the luminal side was measured.
c) The disappearance of 2-PAM from the luminal side was measured in the presence of A.C.D.B.
d) After 30 min perfusion of 4 mM A.C.D.B., the drug solution was washed out with 50 ml of physiologic saline and then the disappearance of 2-PAM from the luminal side was measured.
water transfer ratio: initial *vs.* final concentration ratio of phenol red

In order to further investigate the inhibitory effect of these amine drugs, A.C.D.B. was selected as a model drug. The degree of inhibition of 2-PAM absorption became greater as the initial concentration of A.C.D.B. became higher (Fig. 3). On the other hand, the disappearance of A.C.D.B. was not changed significantly between in the presence and the absence of 2-PAM. Subsequently, in the presence of 1 mM A.C.D.B., the disappearance of 2-PAM at various initial concentrations was investigated. The results are shown in Fig. 1. These values were plotted in Fig. 4 to show the reciprocal of disappearance rate as a function on the reciprocal of initial concentration, using a procedure similar to that proposed by Line-

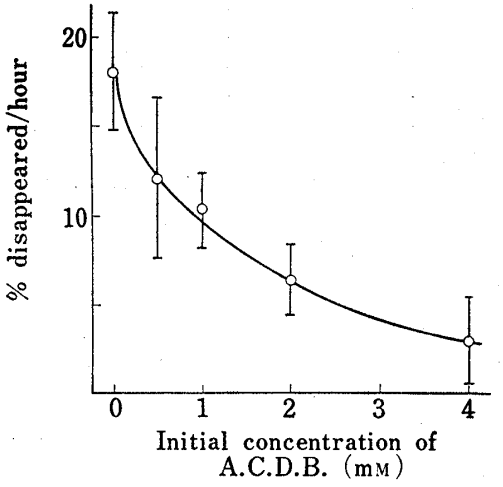

Fig. 3. Effect of A.C.D.B. on the Disappearance of 2-PAM from the Rat Small Intestine

concentration of 2-PAM: 1 mM
Each point represents the mean value of at least five experiments.
Vertical bars indicate s.d.

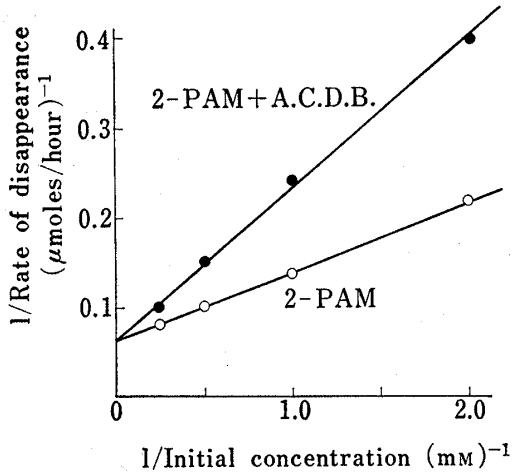

Fig. 4. Lineweaver-Burk Plots for 2-PAM in the Presence and the Absence of A.C.D.B.

concentration of A.C.D.B.: 1 mM
equation to line:
$y=0.0798x+0.0599$ for 2-PAM
$y=0.1708x+0.0626$ for (2-PAM+A.C.D.B.)
correlation coefficient:
0.9996 for 2-PAM
0.9986 for (2-PAM+A.C.D.B.)

weaver and Burk for the analysis of enzyme kinetics. Two straight lines were obtained in the absence and the presence of A.C.D.B. and these lines intersect at the same point on the axis representing reciprocal of disappearance rate. These findings suggest that if the concentration of A.C.D.B. is increased sufficiently the inhibition of its disappearance can be overcome and, therefore, that the competition between 2-PAM and A.C.D.B. is competitive.

To ascertain whether the inhibitory effect by amine drugs on the disappearance of 2-PAM was due to a direct action on the membrane permeability, A.C.D.B. was administered intravenously and the disappearance of 2-PAM from the luminal side was measured. The results (18.8±1.0%/hr) did not significantly differ from the control values (Table I). When the same amount of A.C.D.B. was administered to the luminal side, the disappearance of 2-PAM decreased comparing the control values (Fig. 3). Furthermore, the disappearance of 2-PAM from the small intestine pretreated with 4 mM A.C.D.B. for 30 min did not significantly differ from the control values (Table I). From these findings, it may be concluded that only when 2-PAM and amine drugs exist simultaneously in the luminal side, the absorption of the former is inhibited.

Considering the molecular structures of amine drugs having the inhibitory effect, they have aromatic (or cyclohexyl) and dialkylamine group in common. (β-Phenylethylamine and tyramine are primary amines.)

Other basic drugs having no these groups such as moroxydine and aminopropylon, acidic drugs such as salicylic acid, sulfanilic acid and sulfisoxazole, and neutral drugs such as caffeine and nicotinamide did not affect the absorption of 2-PAM at all. These results are shown in Table II.

Table II. Effect of Basic, Acidic, and Neutral Drugs on the Disappearance of 2-PAM from the Rat Small Intestine

Drug	Concn. (mM)	% disappeared per hour	Water transfer ratio
Control	—	18.1±3.3	0.926±0.023
Moroxydine	2.0	22.9±3.5	0.929±0.021
Aminopropylon	2.0	21.1±2.9	0.934±0.034
Salicylic acid	2.0	18.3±0.6	0.951±0.037
Sulfanilic acid	2.0	19.1±2.7	0.956±0.048
Sulfisoxazole	2.0	19.0±2.9	0.922±0.029
Caffeine	2.0	20.5±2.0	1.031±0.016
Nicotinamide	4.0	21.5±3.3	0.924±0.024

concentration of 2-PAM: 1 mM

Effect of Amine Drug on the Disappearance of A.C.D.B.

It is well known that aromatic and amino group contribute to the interfacial reaction in the biological systems as hydrophobic and hydrophilic part of a molecule respectively. In order to investigate whether drugs having these commom structures interact each other in the membrane transport, the disappearance of the combination of A.C.D.B. and diphenhydramine from the small intestine was studied. Due to the addition of diphenhydramine, the disappearance of A.C.D.B. became smaller compared to the control values. As shown in Fig. 5, these results fitted with the Lineweaver-Burk plots approximately, and the disappearance of A.C.D.B. appeared to be competitively inhibited by diphenhydramine.

Studies on the Mechanism of the Intestinal Absorption of 2-PAM

Neverthless 2-PAM is almost completely ionized in the physiological pH range, the preceding results indicated that the disappearance of 2-PAM from rat small intestine was fairly well and would be absorbed by more than a process of passive diffusion. Therefore, we per-

formed following experiments to elucidate the mechanism of intestinal absorption of 2-PAM. It is well documented that the competition is caused in the membrane transport among the compounds requiring the same energy systems and having similar structures. Then, we investigated whether 2-PAM interacted with the transport systems of amino acids and sugars. The results are shown in Table III. D-Glucose did not influence at all but two amino acids moderately inhibited the 2-PAM absorption. These results indicate that there is a possibility that 2-PAM interacts with amino acids transport system, but the precise mechanism remains uncertain.

To obtain further information, *in vitro* everted sac method was employed. The initial concentration of 2-PAM were equal in the mucosal and serosal side. The concentration ratios of serosal to mucosal after 60 min incubation are shown in Table IV. Similar ratio of L-tryptophan which is actively transported is above 1 as shown in Table V, while the ratios for 2-PAM are far below 1. From these results, it was suggested that 2-PAM was accumulated considerably in the intestinal wall and might not be absorbed actively. That the concentration ratios of 2-PAM were higher in the presence of A.C.D.B. would be attributed to the fact that A.C.D.B. inhibited the uptake of 2-PAM to the intestinal wall.

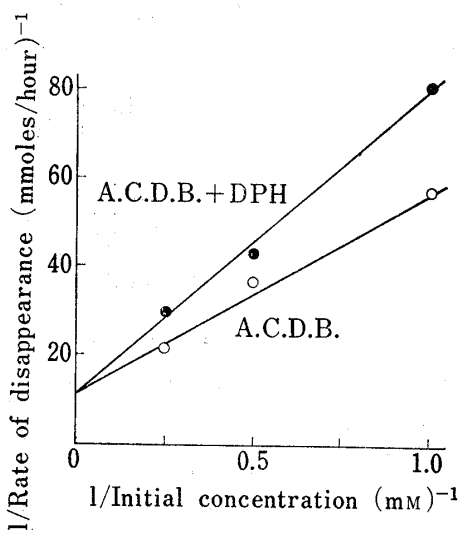

Fig. 5. Lineweaver-Burk Plots for A.C.D.B. in the Presence and the Absence of Diphenhydramine (DPH)

concentration of DHP: 2 mM
equation to line:
$y=46.4x+11.2$ for A.C.D.B.
$y=68.6x+10.9$ for (A.C.D.B.+DPH)
correlation coefficient:
0.9929 for A.C.D.B.
0.9956 for (A.C.D.B.+DPH)

TABLE III. Effect of Amino Acids and Glucose on the Disappearance of 2-PAM from the Rat Small Intestine

Compound	Concn. (mM)	% disappeared per hour	Water transfer ratio
Control	—	18.1±3.3	0.926±0.023
L-Tryptophan	20	13.4±1.8	1.054±0.021
L-Alanine	20	11.3±3.5	0.940±0.037
D-Glucose	280	20.0±0.8	0.997±0.017

concentration of 2-PAM: 1 mM

TABLE IV. Ratio of Concentration of 2-PAM between Serosal and Mucosal Fluid at Equilibrium

Drug composition		Ratio of serosal to mucosal concn.	
Mucosal side	Serosal side	Jejunum	Ileum
2-PAM	2-PAM	0.62±0.11	0.71±0.06
2-PAM+A.C.D.B.	2-PAM+A.C.D.B.	0.89±0.06	0.99±0.01
2-PAM+A.C.D.B.	2-PAM	0.73±0.16	—

drug concentration: 2-PAM (1 mM), A.C.D.B. (4 mM)

In hyperphenylalaninemia, the level of phenylalanine in the circulation has a direct and a specific influence on the intestinal absorption of this amino acid.[29] Intestinal absorption of phenylalanine was inhibited specifically among several neutral amino acids and this in-

Table V. Ratio of Concentration of L-Tryptophan between Serosal and Mucosal Fluid at Equilibrium

Site of intestinal sac	Ratio of serosal to mucosal concn.
Upper jejunum	3.09
Lower jejunum	1.85
Upper ileum	1.16
Lower ileum	1.03

The initial serosal and mucosal concentrations of L-tryptophan were equal (2 mM).

hibitory effect was neither related to the amino acid concentration in the intestinal mucosa nor to the phenylalanine hydroxylase activity of the small intestine. After the intravenous injection of 2-PAM (10 mg per rat), the disappearance rate of this drug from the intestinal lumen was measured and proved not to be altered, so it seemed probable that the amount of 2-PAM in the circulation would not contribute to the saturation phenomena in the absorption process.

Recently, several observations that the relatively large amount of quaternary ammonium compounds is bound to the intestinal tissues have been obtained.[4,30] As the drug absorbed means the drug disappeared from the intestinal lumen in the perfusion experiments, it is impossible to determine accurately the amount absorbed. Accordingly, the distribution of 2-PAM between the gut wall and the circulation was examined by *in situ* loop method at the jejunal site. Because circulatory uptake was determined by the difference between the initial dose and the total amount of final luminal residue and of tissue binding, it was determined by *in vitro* everted sac method whether there was metabolic destruction of 2-PAM in the loops during the experiments. The recovery of 2-PAM was more than 96%. Thus, there seems to be little drug loss due to metabolism or destruction. The time course of the distribution of 1.32 mg of 2-PAM was examined for 90 min. The results are shown in Fig. 6. The decrease of luminal content of 2-PAM proceeded exponentially till 60 min and the uptake to the gut wall remained constant after 15—30 min.

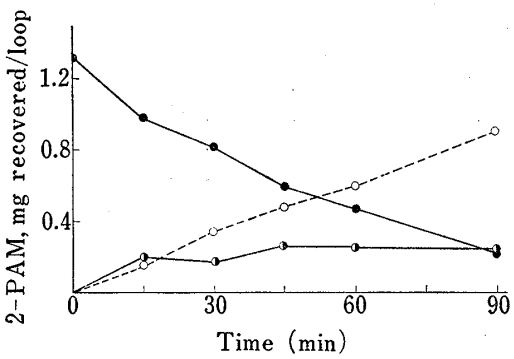

Fig. 6. Time Course of 2-PAM Distribution in Jejunal Loop

2-PAM dose: 1.32 mg per loop
key: ● residue in intestinal lumen
○ circulatory uptake
◑ intestinal tissue binding

From these results, it is suggested that 2-PAM is bound to the intestinal wall and the rate of uptake to the intestinal mucosa or the rate of delivery to the circulation seems to control the transfer of 2-PAM.

Studies on the Mechanism of the Inhibition of 2-PAM Absorption by A.C.D.B.

To investigate whether nine amine drugs inhibited specifically the disappearance of 2-PAM, the intestinal absorption of quinine and L-tryptophan in the presence of A.C.D.B. was studied by perfusion method: the former transferred by passive diffusion and the latter transferred by active transport. The results are shown in Table VI. A.C.D.B. did not seem to exert any effect on the disappearance of both compounds. These results suggest that the inhibitory effect of A.C.D.B. and other amine drugs is brought on the specified drugs.

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Table VI. Effect of A.C.D.B. on the Disappearance of Quinine and L-Tryptophan from the Rat Small Intestine

Drug	Concn. (mM)	% disappeared[a)]	Water transfer ratio
		None	
Quinine	0.4	29.7±4.7	0.897±0.021
L-Tryptophan	1.0	69.5±9.1	—
		with A.C.D.B.	
Quinine	0.4	30.6±3.7	0.923±0.018
L-Tryptophan	1.0	65.6±7.8	—

a) quinine: % disappeared per hour, L-tryptophan: % disappeared per 20 min
concentration of A C.D.B.: 2 mM for quinine, 4 mM for L-tryptophan

Table VII. Effect of A.C.D.B. on 2-PAM Distribution by Everted Sacs of the Rat Small Intestine

	None	with A.C.D.B.	P Value
No. of expts.	6	6	—
Wet gut weight (gr)	0.70±0.13	0.73±0.06	n.s.
Final serosal volume (ml/hr·sac)	1.21±0.18	0.88±0.08	0.005
Serosal 2-PAM transfer (μmoles/hr·sac)	0.22±0.05	0.20±0.03	n.s.
Gut wall 2-PAM (μmoles/hr·sac)	0.70±0.09	0.56±0.10	0.05

The everted sacs contained initially 0.80 ml standard Ringer solution and were incubated for 60 min under aerobic conditions in 8.0 ml standard Ringer solution containing 2 mM 2-PAM. A.C.D.B. (4 mM) was added to the mucosal fluid.

Subsequently, in order to investigate which step in the membrane transport of 2-PAM was affected by A.C.D.B., *in vitro* transfer experiments were carried out in which 2-PAM was initially present in the mucosal fluid. The results are shown in Table VII. These results indicate that A.C.D.B. inhibits the accumulation of 2-PAM to the gut wall and the water transfer to the serosal side significantly.

Further, the effect of A.C.D.B. on 2-PAM absorption was examined employing *in situ* loop method. The dose of A.C.D.B. was 1.74 mg per loop. The results are shown in Fig. 7. The luminal residue of 2-PAM in the absence of A.C.D.B. is dependent on initial doses. This seems to agree with the information in the perfusion experiments. While the percentage of tissue binding seems not to change with the concentration, A.C.D.B. would affect the luminal residue but not the tissue binding of 2-PAM. Metabolic inhibitor, potassium cyanide, did not affect the 2-PAM distribution. The discrepancy between *in vitro* everted sac and *in situ* loop method may be due to the differences in the limiting steps in membrane transfer process.

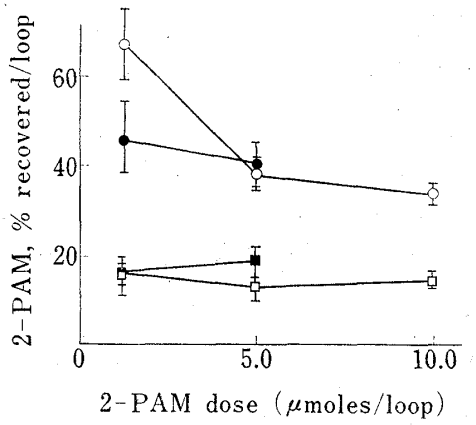

Fig. 7. Effect of A.C.D.B. on the Distribution of 2-PAM in the Rat Jejunal Loop

A.C.D.B. dose: 5.0 μmoles per loop
time: 30 min
key: total sorption (sum of tissue binding and circulatory uptake)
○ 2-PAM, ● 2-PAM with A.C.D.B.
tissue binding
□ 2-PAM, ■ 2-PAM with A.C.D.B.

Studies of the Absorption Mechanism by Isolated Intestinal Epithelial Cells

In order to investigate the effect of A.C.D.B. on the intestinal absorption of 2-PAM in detail, the uptake of 2-PAM to isolated intestinal epithelial cells was measured. Viable

preparations of epithelial cells were often used to clarify the properties of intestinal transport of amino acids and sugars.[31] The major advantages of the epithelial cells over intestinal segments in studying transport mechanisms are as follow: there is less variability among preparations; there are only two compartments, cells and medium; there is a greater proportion of absorptive to nonabsorptive portion. Isolated intestinal epithelial cells were obtained by the method of Reiser and Christiansen.[23] Microscopic examination of the sections of residual intestine revealed that the cells at the crypts still remained but the cells at the upper portion of villi were removed entirely. The uptake of 2-PAM by isolated intestinal epithelial cells is shown in Table VIII. Considering the amount of cells to be proportional to the amount of protein,[23] 2-PAM uptake was calculated per mg protein. 2-PAM uptake was reduced significantly in the presence of A.C.D.B. From these results, it was suggested that A.C.D.B, brought the inhibitory effect in the process where 2-PAM was taken up into the epithelial cells.

Table VIII. Effect of A.C.D.B. and 2-PAM Concentration on the Uptake of 2-PAM by Isolated Intestinal Epithelial Cells

Concn. of 2-PAM (mM)	Concn. of A.C.D.B. (mM)	2-PAM Uptake (nmoles/ mg protein)
2.0	—	11.2±1.6
5.0	—	23.4±1.4
10.0	—	44.3±3.2
2.0	4.0	7.8±0.7[a]

a) significant as compared to the value for 2-PAM in the absence of A.C.D.B. ($p<0.005$)

From above experiments, it was concluded that the intestinal absorption of 2-PAM and A.C.D.B. was inhibited in the presence of several amine drugs. Among these drugs, physicochemical interaction could not be observed. As shown in Table I, intravenous administration of A.C.D.B. or pretreatment of the luminal side by A.C.D.B. solution gave no influence to the absorption of 2-PAM. From these facts their inhibitory effects were not attributed to the alteration of the permeability of membrane or blood cappillary. Furthermore, it was shown that the inhibitory effect of amine drugs had drug specificity, because the intestinal absorption of quinine and L-tryptophan did not significantly differ in the presence of A.C.D.B. From the results of *in vitro* experiments, the accumulation of 2-PAM to gut wall and the uptake to epithelial cells were shown to be inhibited by amine drugs. Since the pharmacologic action of amine drugs is so varied and the drugs with opposite pharmacologic action inhibit 2-PAM absorption, it is supposed that the molecular structures which amine drugs have in commom (phenylalkylamine) may related to the inhibitory effect. In order to elucidate these mechanism, further studies are needed.

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31) B.K. Stern and W.E. Jensen, *Nature*, **209**, 789 (1966); G.A. Kimmich, *Biochemistry*, **9**, 3669 (1970); J.W. Halliday and L.W. Powell, *Clin. Chim. Acta*, **43**, 267 (1973).