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Absorption and Excretion of Drugs. XLI.[1] Studies on the Gastrointestinal Absorption of 2-Pyridine Aldoxime Methiodide and Its Derivatives. (1)

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The gastrointestinal absorption of pyridine aldoxime methiodides has been studied in rats using single loop and perfusion techniques.

Contrary to their apparent physicochemical properties, these compounds were absorbed from the intestine to an appreciable extent but not from the stomach and the rectum within one hour. The absorption from the intestine became saturated when the concentration of the drugs increased and one will inhibit the absorption of the other. The presence of a metabolic inhibitor in the drug solution had no effect on the absorption of these compounds. Despite the complexity of the systems used, a straight line relationship between the reciprocal of absorption rate and the reciprocal of initial concentration was obtained for each compound studied.

Although passive transfer across a lipoid–pore boundary adequately describes the gastrointestinal absorption of many foreign organic compounds, it does not explain the transfer of certain strong organic electrolytes. Generally the rates at which organic anions and cations cross the intestinal epithelium are extremely small compared with the rates of passage of lipoid soluble, uncharged molecules.

Highly ionized bases such as quaternary ammonium compounds are poorly and irregularly absorbed from the gut. The therapeutic zone of these potent compounds are sometimes narrow. In most instances small doses have no effect and the therapeutic responses is achieved with a relatively narrow dose range. Therefore, such characteristics sometimes becomes a matter of great therapeutic importance as this poor absorption causes great variations between different individuals and day to day variations in the same individual.

In their studies on the mechanism of intestinal absorption of quaternary ammonium compounds, Levine, *et al.*[3] suggested that of the many compounds studied, the mono-quaternary pyridinium aldoximes, but not bis-aldoximes, were the only exceptions to the general rule of the poor absorbability of quaternaries. Earlier, they suggested that a fraction of tissue, the phosphatidepeptide fraction might play an important role in absorption enhancement of benzomethamine.[4] Contrary, other reports[5] from the same laboratory revealed that the formation of a non-absorbable complex with mucin reduced the absorption of monoquaternaries from the intestine. In spite of all the available data, no definite information is available pertaining to the mechanism of the absorption of highly ionized cationic drugs from the intestine.

1) Part XL: K. Kakemi, H. Sezaki, S. Muranishi, and Y. Tsujimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1641 (1969).
2) Location: *a*) *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto*; *b*) *Kashima-cho, Higashiyodogawa-ku, Osaka.*
3) R.R. Levine and G.M. Steinberg, *Nature*, **209**, 269 (1966).
4) *a*) R.R. Levine and E.W. Pelikan, *J. Pharmacol. Exptl. Therap.*, **131**, 319 (1961); *b*) R.R. Levine, *Arzneimittel Forschung*, **16**, 1373 (1966).
5) R.R. Levine, *J. Pharmacol. Exptl. Therap.* **131**, 328, (1961); *b*) R.R. Levine, J. Weinstock, C.S. Zirkle, and R. Mclean, *ibid.*, **131**, 334 (1961).

Because our ultimate goal has been the modification of rate of the gastrointestinal absorption of dissolved, poorly absorbable substances and to develop superior dosage forms for such compounds, we wished to study mechanism of absorption of such drugs over a broad range of biopharmaceutical conditions.

This paper reports a study of the absorption of highly ionized pyridine aldoxime methiodide isomers (PAM iodide) from the gastrointestinal tract of the rat.

Contrary to their apparent physicochemical properties, PAM iodide were absorbed from the rat intestine to an appreciable extent but not from the stomach and the rectum. The absorption from the intestine became saturated when the concentration of the drugs were raised high enough and one will inhibit the absorption of the other.

Experimental

Material——PAM iodide were prepared from corresponding pyridine aldehyde according to Wilson's method.[6] Other chemicals used were of analytical reagent grade.

Animals——Male Wistar rats weighing 140 to 170 g were used for the perfusion procedures and male Sprague–Dawley rats weighing 300 to 350 g were used for the single-loop absorption experiments.

Degradation of PAM Iodide——Stability of PAM iodide was investigated at pH values 3 to 8 and at 37°. Buffer solutions were placed in thermostatically controlled bath, and allowed to reach the temperature of the bath. A volume of PAM iodide solution was then added to give the reaction mixture a concentration of 2 mM. The decomposition of PAM iodide was determined for three hours by periodically removing and assaying samples.

Determination of Partition Coefficient——Mutually saturated solvents were used throughout. The pH of drug solutions containing 0.5 to 2.5 mM of PAM iodide was adjusted by means of either 0.1 N NaOH or HCl. Five milliliter aliquots of a drug solution were equilibrated with equal volumes of organic solvent and kept in a constant temperature bath at 37°. The mixture was well shaken to reach equilibrium and PAM iodide content in aqueous phase was determined and apparent partition coefficients calculated. In the determination of the apparent partition coefficient of PAM iodide as a function of PAM iodide concentration, solutions containing 2.5 to 12.5 mM were prepared. One milliliter of the solution was pipetted into a 25 ml test tube which contained 4 ml of citrate buffer solution and 5 ml of organic solvent. The solutions were kept in a constant temperature bath at 37°, and after equilibration, the phases were separated and assayed.

Analytical Methods——Bathochromic shift of PAM iodide in alkaline solution, as shown in Fig. 1, was used for the determination. All spectrophotometric analyses were performed with a Hitachi 139 spectrophotometer.

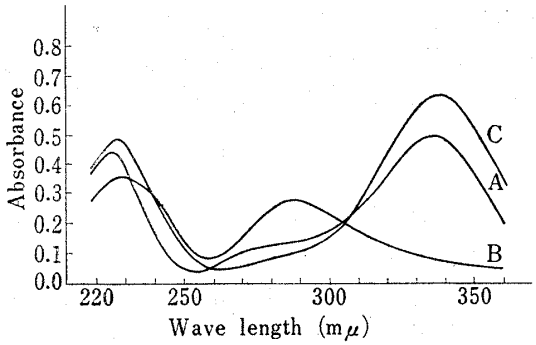

Fig. 1. Ultraviolet Absorption Spectra of PAM Iodide

PAM iodide 2.5×10^{-5}M in 0.1M NaOH solution
A: 2-PAM iodide B: 3-PAM iodide
C: 4-PAM iodide

For the determination of PAM iodide in the gastrointestinal perfusate, 1 ml of sample solution was pipetted into a 12 ml glass-stoppered centrifuge tube which contained 5 ml of isoamyl alcohol saturated with distilled water and 4 ml of 0.1 N HCl. After shaking vigorously for 30 minutes and centrifuging, 3 ml aliquots of aqueous phase was transferred to a test tube containing 9 ml of 0.2 N NaOH. To minimize alkaline degradation of PAM iodide, the absorbance of the mixture was determined immediately at 336 mμ, 289 mμ, and 336 mμ for 2, 3, and 4-PAM iodide, respectively against an appropriate blank.

For the determination of PAM iodide in the gut homogenate preparations, 3 ml of homogenate sample was added to a glass-stoppered 12 ml centrifuge tube which contained 3 ml of 2 N trichloroacetic acid. After shaking vigorously and centrifuging, PAM iodide in the supernatant was determined by the method for the gastrointestinal perfusate.

6) S. Ginsburg and I.B. Wilson, *J. Am. Chem. Soc.*, **79**, 481 (1957).
7) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Explt. Therap.*, **120**, 528 (1957).

Absorption Experiments——(1) Absorption from the Stomach: The *in situ* ligation technique developed by Schanker, *et al.*[7] was used. Animals were maintained under urethane anesthesia for the entire course of the experiment.

(2) Absorption from the Small Intestine (Single-loop Method): The degree of intestinal absorption was measured using *in situ* single intestinal loop preparations. With the rats under light urethane anesthesia, the intestine was exposed by a mid-line incision and the proximal ligature placed about 15 cm from the pylorus. The length of the loop was about 20 cm. Both ligatures were cannulated with silicon tubings. No major blood vessels were occuluded by these ties. 0.9% NaCl solution was injected into the loop until washings became clear. One milliliter of drug solution was injected into the loop from the proximal end. The ligature was secured and the incision closed. After an hour, the loop of the gut was removed for chemical analysis and determination of its quantity of unabsorbed PAM iodide. After washing out the contents of the lumen, 0.9% NaCl solution was added and the gut preparation was homogenized and the mixture was used for the determination of PAM iodide bound on the surface of the intestinal membrane or within the intestinal wall. In the case of the determination of PAM iodide transferred into blood, mesenteric blood vessels were cannulated to collect the heparinized blood from the intestine. The blood preparation was collected up to 30 minutes.

(3) Absorption from the Small Intestine (Perfusion Method): Male rats, weighing 130—160 g, were fasted overnight prior to the experiments but allowed free access to water. The animals were anesthesized with urethane and the small intestine was cannulated for *in situ* recirculation. The intestine was first washed with about 50 ml of a 0.9% NaCl solution maintained at 37°, and then with 25 ml of test solution. After the test solution was forced out, the tubings attached to the inflow and outflow cannulae were transferred to a flask containing 25 ml of test solution. This volume was then continuously circulated for one hour through the intestine at a rate of 5 ml/min. In the case of the study of the effect of 2, 4-dinitrophenol addition on the absorption of PAM iodide, 30 minutes, instead of one hour, absorption period was used so as to maintain the enough concentration of the rapidly absorbable inhibitor throughout the experiment. Further experimental detail can be found in previous paper from this laboratory.[8]

(4) Absorption from the Rectum: The procedure used was the same as that reported in the earlier paper[9] from this laboratory except that animals were anesthesized with urethan and 20 ml of drug solution was circulated through the rectum for one hour at 37° with the flow rate of 5 ml/min.

(5) Test Solution: In order to eliminate complications which may arise from a buffer solution, a 0.9% NaCl solution rather than a buffer solution was employed to prepare PAM iodide solutions. Change in pH during absorption experiment was negligibly small. No volume correction indicator was used except in the stomach, where phenolred was added to the test solution. Sample solution was washed out with a 0.9% NaCl solution to make the volume of 100 ml in the case of perfusion experiment.

Results and Discussion

Stability of PAM Iodide in the Absorption Experiment

Ellin, *et al.*[10] reported the decomposition of PAM iodide at pH values 0.5 to 13 and at temperatures from 37° to 87°. Within the pH range studied, however, degradation of PAM iodide was not observed. It may be concluded that no degradation of PAM iodide is taken place under the condition of the absorption experiment.

Apparent Partition Coefficient

Values of pK_a assigned for oxime group of 2, 3, and 4-PAM iodide are 8.0, 9.2, and 8.6, respectively. Therefore, PAM iodide exhists largely as a mono cation at the pH range examined (3—8) and transfer of PAM iodide to the organic phase could hardly be observed. This tendency did not change with increase or decrease of the initial concentration of PAM iodide or by the addition of excess amount of anions such as chloride, bromide, or iodide.

Absorption of PAM Iodide

Intestinal absorption of PAM iodide was determined by *in situ* perfusion technique. Contrary to the poor lipoid solubility, the rates at which 2-PAM iodide cross the small intestine was not so slow as shown in Fig. 2.

8) *a*) K. Kakemi, T. Arita, and S. Ohashi, *Yakugaku Zasshi*, **82**, 348 (1962); *b*) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, (Tokyo), **12**, 421 (1964).
9) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861 (1965).
10) R.I. Ellin, J.S. Carlese, and A.A. Kondritzer, *J. Pharm. Sci.*, **51**, 141 (1962).

The intestinal absorption of this highly ionized, lipoid insoluble compound is also demonstrated in Fig. 3 in terms of amount absorbed in one hour as a function of its initial concentration.

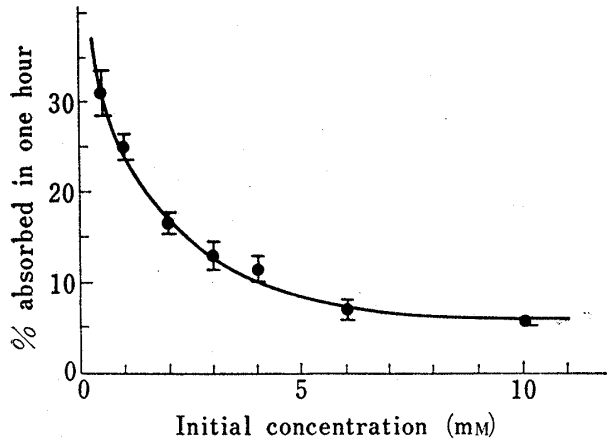

Fig. 2. Absorption of 2-PAM-iodide from the Rat Small Intestine by *in situ* Perfusion Technique

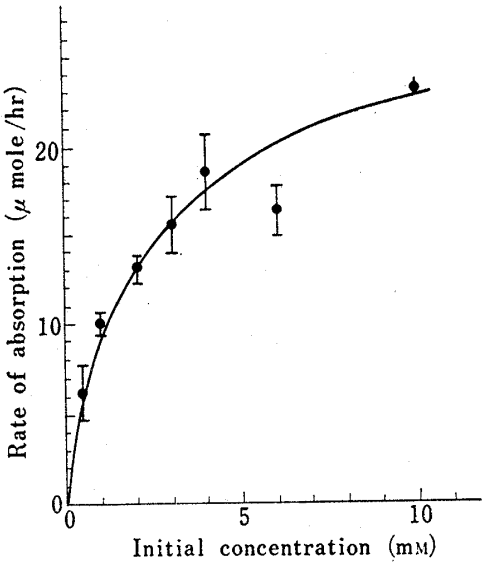

Fig. 3. Rate of One Hour Absorption of 2-PAM-iodide from the Rat Small Intestine by *in situ* Perfusion Technique as a Function of initial Concentration

It is interesting to note that contrary to the studies of Levine and Pelikan on the absorption of benzomethamine from the rat intestine, no proportionality was observed between the initial concentration of the drug and absorption of 2-PAM iodide. For example, at a concentration of 1 mM, % absorbed in one hour was 25 and at 6 mM, this value goes down to about 7. Similar tendency was observed for 3, and 4-PAM iodide as shown in Table I.

Table I. % Absorbed of PAM-iodide in 60 Minutes Perfusion Experiment from Solutions of Various Concentrations

Initial concentration (mM)	2-PAM-I	3-PAM-I	4-PAM-I
0.5	31.1	40.0	32.5
1.0	24.9	35.8	24.4
2.0	16.4	25.3	16.5
3.0	13.0	21.9	14.0
4.0	11.6	19.0	11.7

In the stomach and in the rectum of the rat, however, no absorption of PAM iodide was observed in one hour. Hence, this apparent saturation in absorption rate as well as absorption of such highly ionized compounds may be a specific characteristics in the absorption from the small intestine. Although addition of metabolic inhibitor, 2,4-dinitrophenol, to the perfusion solution exerted any influence on the absorption rate of PAM iodide as shown in Table II, it is evident that saturability together with site specificity in the absorption of PAM iodide can not be explained in terms of simple diffusion of uncharged molecules across a lipoidal barrier.

That PAM iodide disappeared from the perfusion solution was transferred to serosal side and entered into blood stream was confirmed by the single-loop absorption experiment.

Table II. Effect of 2,4-Dinitrophenol on the 30 Minutes Absorption of 2-PAM-iodide

	Initial concentration of 2,4-DNP (mM) 0	0.1	0.5	1.0	5.0
Single-loop method[a] (10.0 mM 2-PAM-I) (%)	14.1	14.1	13.7	15.2	15.4
Perfusion method (2.0 mM 2-PAM-I) (%)	7.5	7.7	7.7	—	—

a) Values do not include adsorption to the intestinal wall.

One milliliter of 10 mM PAM iodide solution was injected quantitatively into an intestinal loop of the rat and after one hour, drug remaining in the lumen, within the intestinal wall, and transported into the blood was determined. As shown in Table III, considerable amount of PAM iodide, disappeared from the injected solution, was recovered in blood.

Table III. Distribution of PAM-iodide in the Various Compartments after 30 Minutes Single-loop Absorption Experiment

	Lumen	Intestinal wall	Blood
2-PAM-I (%)	73.3	11.9	14.8
3-PAM-I (%)	71.0	15.0	14.0

It has been found experimentally that, for many systems classified for specialized transport, the unidirectional movement across the intestinal wall is given by an expression of the form of Michaelis Menten type equation in enzyme kinetics. This was applied to the absorption data of PAM iodide. The values were plotted to show the reciprocal of transport rates as a function of reciprocal of initial concentrations of PAM iodide. The graphs in Fig. 4 reveal for each PAM iodide studied a straight line relationship between the reciprocal of absorption rate and the reciprocal of initial concentration indicating that the absorption kinetics of PAM iodide are consistent with Michaelis Menten kinetics.

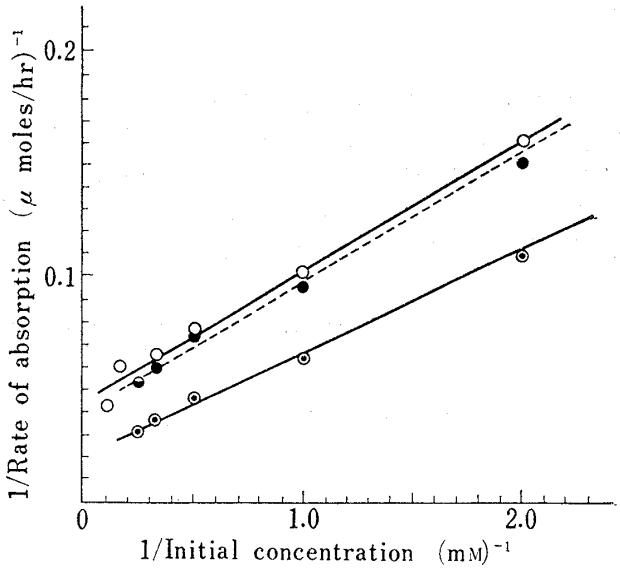

Fig. 4. Lineweaver-Burk Plots for PAM-iodide

—○—: 2-PAM-iodide —⊙—: 3-PAM-iodide
—●—: 4-PAM-iodide

Values were determined from the graphs for V_{max}, the apparent limiting rate of absorption, and K_m, the concentration of PAM iodide at which half the maximum rate is attained. These are summarized in Table IV.

Table IV. Values of K_m and V_{max} for PAM-iodide

	V_{max} (10^{-5} mole/hr)	K_m (M^{-1})
2-PAM-I	2.27	1.31
3-PAM-I	5.08	2.60
4-PAM-I	2.34	1.31

Values of K_m suggest that the apparent affinity of 3-PAM iodide for the absorptive membrane or specialized transport mechanism was weaker than those for 2, and 4–PAM iodide, whereas the values of V_{max} for 3-PAM iodide was the largest among these derivatives.

Since general characteristics of specialized transport process involves competitive phenomenon by the presence of molecules structurally analogous to the permeant considered, inhibitory effect of each position isomer of PAM iodide was examined. The absorption of 2-PAM iodide from solutions of various concentration was measured in the presence of 2 mM solution of 3-PAM iodide. The results in Fig. 5 was compared with the absorption of 2-PAM iodide alone. The absorption of 2-PAM iodide was inhibited by the presence of 3-PAM iodide, the most rapidly absorbable derivatives among the three. Similar plot was obtained with the absorption of 4-PAM iodide in the presence of 3-PAM iodide as shown in Fig. 6. Contrary, absorption of 3-PAM iodide, the most rapidly absorbable isomer, was inhibited slightly by the presence of either 2, or 4-PAM iodide.

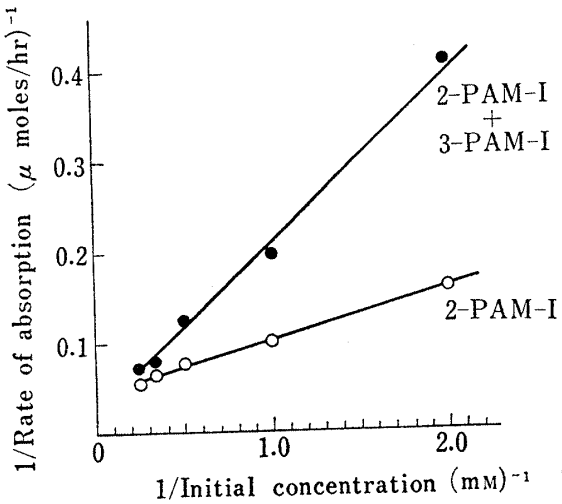

Fig. 5. Lineweaver-Burk Plots for 2-PAM-iodide in the Presence and Absence of 3-PAM-iodide

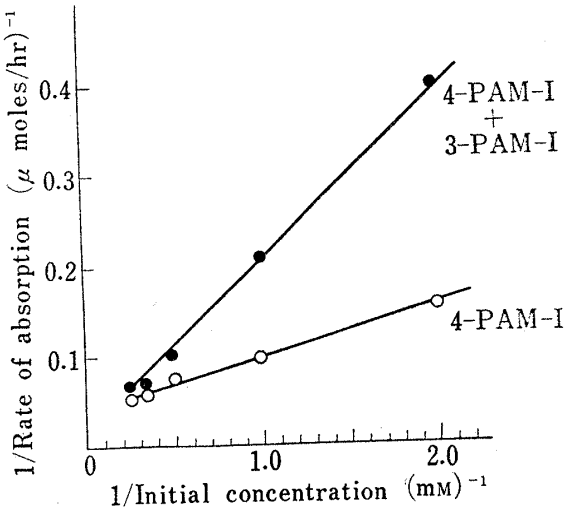

Fig. 6. Lineweaver-Burk Plots for 4-PAM-iodide in the Presence and Absence of 3-PAM-iodide

The values obtained for these Michaelis Menten kinetics are dependent on the experimental techniques employed, and the complexity of the systems used adds to the difficulty of interpretation.

It must be remembered, however, that such observations do not demonstrate the nature of the transport mechanism, and is nothing more than an indication of a saturable rate–limiting step in the process of transport, a step which might be enzymatic, or which might be a process such as combination with, or absorption to some ionic or nonionic component of the adsorptive membrane. Anyway, compounds other than those of physiological importance are absorbed from the intestine at a rate faster than could reasonably be expected from their apparent physicochemical chracteristics and that the saturation in absorption rate is attained relatively small concentration range are of interesting from the standpoint of the movement of molecules across the intestinal wall as well as the standpoint of biopharmaceutics.

It is also interesting in view of the recent findings by Kondritzer.[11] In the human plasma level analysis, they revealed that ten–fold increase in dosage of 2-PAM increased the peak plasma levels of oxime only 3.5-fold and a logarithmic equation was presented between peak

11) A.A. Kondritzer, P. Zvirblis, A. Goodman, and S.H. Paplanus, *J. Pharm. Sci.*, **57**, 1142 (1968).

plasma levels and oral dosage. Since dissolution characteristics of the compound in the gastrointestinal tract dose not seem to be a limiting factor in the absorption of the drug from the human intestine, this peak plasma level-dose relationship could be interpreted in many different factors in the body such as distribution, metabolism, and excretion. However the possiblity of a mediation of a specialized transport mechanism in the gastrointestinal absorption of the drugs can not be ruled out.