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Intestinal Absorption Mechanisms of Ampicillin Derivatives in Rats. I.[1] Intestinal Absorption of Ampicillin Derivatives

Katsumi Miyazaki, Osamu Ogino,[2a] Masahiro Nakano, and Takaichi Arita[2b]

Pharmacy, Hokkaido University Hospital[2a] and Faculty of Pharmaceutical Sciences, Hokkaido University[2b]

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In order to investigate the reasons why the body fluid concentrations of pivampicillin and amoxicillin were higher than those of ampicillin after administration of the drugs to rats and humans, the mechanisms of intestinal absorption of these antibiotics in rats were examined using the new fluorometric determination method. In these experiments, it was found that the higher lipid solubility and the greater dissolution rate of pivampicillin are the main reasons for the higher body fluid concentrations of ampicillin after administration of pivampicillin. However, the higher body fluid concentrations of amoxicillin could not be explained on the basis of these physicochemical properties. In the studies using the rat small intestinal segments, *i.e.* the everted intestine, everted sac and the intact intestinal loop, greater binding to the mucosa of amoxicillin than of ampicillin was observed. This observation may provide an explanation for the difference in body fluid concentrations of these two drugs. These two amphoteric penicillins may be absorbed from the rat small intestine by passive diffusion mechanism.

Keywords——ampicillin derivatives; physicochemical properties; dissolution; drug absorption; intestinal loop; accumulation; everted sac; passive diffusion

On the intestinal absorption of aminobenzylpenicillins, most of the published reports[3] deal with the relative superiority or inferiority of their absorption as shown in experiments on their blood levels and urinary excretion measured by the microbiological method and there have been only a few reports[4] so far published on the mechanisms of permeability of these penicillins through the membrane of the digestive tract. However, it is considered very important to clarify the mechanisms of membrane permeability of amphoteric penicillins which are said to be absorbed well in spite of their amphoteric ion structure and very low lipid solubility.

In previous papers,[5] the authors investigated the urinary excretion and blood levels of unchanged antibiotics and metabolites in man and rats after administration of ampicillin, pivampicillin and amoxicillin using a chemical separation assay method. In these experiments, marked differences in the body fluid concentrations of these three antibiotics were observed.

This work was undertaken to investigate the factors influencing the marked differences in the body fluid concentrations of these antibiotics. The membrane permeability of ampicil-

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2) Location: *a*) *Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo*; *b*) *Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo.*

3) R.N. Brogden, T.M. Speigh, and G.S. Avery, *Drugs*, **7**, 326 (1974); P.A. Pamela, A. Hunter, L. Mizen, and G.N. Rolinson, *Antimicrob. Ag. Chemother.*, **1971**, 416; H. Neu and E.B. Winshell, *ibid.*, **1970**, 423; *etc.*

4) S.C. Penzotti, Jr. and J.W. Poole, *J. Pharm. Sci.*, **63**, 1803 (1974).

5) *a*) K. Miyazaki, O. Ogino, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 1910 (1974); *b*) K. Miyazaki, O. Ogino, M. Nakano, and T. Arita, *ibid.*, **23**, 178 (1975); *c*) K. Miyazaki, O. Ogino, H. Sato, M. Nakano, and T. Arita, *ibid.*, **25**, 253 (1977).

lin and of amoxicillin was compared closely, investigated and discussed on the basis of adsorption of these drugs onto the rat small intestinal membrane, and the transfer mechanisms of these drugs across the small intestine were also discussed.

Experimental

Materials and Reagents——Anhydrate form of ampicillin (potency: 1025 μg/mg) was kindly supplied by Takeda Chemical Industries, Ltd., Osaka, Japan. Pivampicillin hydrochloride (pure powder) was kindly supplied by Sankyo Co. Ltd., Tokyo, Japan. Trihydrate form of amoxicillin (potency: 814 μg/mg) was kindly supplied by Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan. Aminobenzylpenicilloic acid solution was prepared with 1 N NaOH. All the reagents were of special grade, and were prepared with redistilled water. Quinine sulfate solution, which is the standard fluorescence solution used in this experiment, was prepared as described previously.[5a]

Apparatus——Fluorescence intensity was measured on a Hitachi spectrofluorometer, model 203, equipped with a Xenon lamp.

Animals——Wistar male rats (250—300 g) were used as the experimental animals. The rats were subjected to the experiment after they had been anesthetized with ether and urethane.

Procedure for Determination of Apparent Partition Coefficients——Solutions of exactly 0.2 mM and 1 mM of ampicillin, amoxicillin, pivampicillin and aminobenzylpenicilloic acid were prepared using an isotonic buffer of pH 5.0, 6.4, and 7.4. Exactly 5 ml of butanol or chloroform was placed into each of two 30 ml glass-stoppered test tubes, and exactly 5 ml of the previously prepared drug-buffer solution was added to each of the two test tubes. The test tubes were placed into a metabolic shaking incubator, regulated at 37°±0.1°, and then the tubes were shaken according to the method described by Purich, *et al.*[6] When equilibrium was achieved, the concentration in the aqueous phase and the control sample was determined.

Procedure for Dissolution Studies——a) Dissolution Behavior of Capsule Preparation: The dissolution rate of the one capsule (250 mg) of ampicillin, amoxicillin and pivampicillin was measured by the rotating basket method of the NF XIII (Method I)[7] and by the beaker method.[8] In these dissolution procedures, the basket and the stainless steel three-bladed propeller were rotated at a rate of 50 rpm, respectively. The capsules of ampicillin and amoxicillin were commercial capsules and pivampicillin was loosely packed in a gelatin hard capsule (JP 8 no. 0).

b) Dissolution Behavior of Compressed Disk: The disk of ampicillin and amoxicillin powder was prepared by compressing 100 mg of each sample under a pressure of 200 kg/cm^2 for 10 minutes in 1.3 cm diameter die according to the method described by Arita, *et al.*[9] The disk was attached to one end of a 1.2 cm diameter glass tube with adhesives. The tube was introduced into the beaker containing 100 ml of redistilled water or pH 1.2 hydrochloric acid solution at 37°, being stirred rapidly by means of a magnetic stirring bar.

In these dissolution procedures, a 1 ml of sample solution was removed at a suitable time intervals using a pipette with a cotton filter.

Permeation through the Small Intestine——The everted intestine, everted sac and loop were prepared using the small intestine of rats and were used in the experiments on membrane permeation and absorption of the antibiotics. Drug solutions were prepared using isotonic buffer solutions with a pH of 5.0, 6.4, and 7.4, and modified Ringer's solution[10] with a pH of 7.4 (potassium concentration: 13 mM).

a) Everted Intestine: In the experiments on membrane permeability of the antibiotics through various segments of the small intestine, the duodenum, proximal jejunum and distal ileum, 10 cm long sections of each were removed quickly, washed through with the normal saline and everted. The everted intestine was bathed in 80 ml modified Ringer's solution containing 50 μg/ml of each of the antibiotics, and the mucosal fluid was continually gassed with 95% O_2 and 5% CO_2 and maintained at 37°. During the experiment (1 hr), a constant circulation of the serosal modified Ringer's solution (80 ml, without drug) was maintained at a flow rate of 3 ml per minute.

b) Everted Sac: The everted sac (10 cm long) from the proximal jejunum of rat, prepared according to the method of Wilson and Wiseman,[11] was placed in 100 ml Erlenmyer flask with 50 ml of modified Ringer's solution containing ampicillin or amoxicillin. The flask was gassed with 95% O_2 and 5% CO_2 and maintained at 37°. The serosal initial volume was 1.0 ml. At the end of the incubation period (1 hr), each sac was emptied into a 10 ml volumetric flask and the 0.5 ml of serosal fluid was diluted with redistilled water.

6) E.D. Purich, J.L. Colaizzi, and R.I. Poust, *J. Pharm. Sci.*, 62, 545 (1973).
7) The National Formulary XIII, 1970, 802.
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11) T.H. Wilson and E.W. Wiseman, *Biochim. Biophys. Acta*, 123, 116 (1954).

c) Loop: A single loop from the upper small intestine was prepared 7 cm from the pylorus according to the method of Levine, *et al.*[12] The bile duct was ligated. After washing the loop gently with 10 ml of the various pH buffer solution (without drug) used in this experiment, 1 ml of the drug solutions of ampicillin or amoxicillin (50 μg/ml) was injected into the loop. After 1 hr, before the animal was killed, the loop was removed and emptied into a 25 ml volumetric flask, and the mucosal side of the loop was throughly rinsed with redistilled water to a volume of 25 ml.

Accumulation of Ampicillin and Amoxicillin by Small Intestinal Slices——Accumulation of ampicillin by the digestive tract membrane was compared with that of amoxicillin by preparing slices of thickness 0.5 cm from the everted intestine of rats (proximal jejunum, 0.5 g wet weight).[13] The slices were placed in 100 ml Erlenmeyer flasks with 50 ml of modified Ringer's solution which was 8×10^{-5} M with respect to ampicillin and amoxicillin, and the flask was gassed with 95% O_2 and 5% CO_2 and maintained at 37°. At the end of the incubation period (1 hr), each of the slices was rinsed with 30 ml of normal saline and blotted with filter paper carefully to remove the adhering moisture. Five ml of homogenate was then prepared in a teflon homogenizer with redistilled water, and both the homogenates and the bathing solutions were used for determination.

Furthermore, in other experiments, 2,4-dinitrophenol (DNP) and sodium azide (NaN_3) were added to each bathing fluid to estimate the effect of metabolic inhibitors on the accumulation of ampicillin and amoxicillin by the intestinal membrane.

Measurement of Percentage Binding of Ampicillin and Amoxicillin to the Mucosa of the Small Intestine——A mucosal preparation of rat upper intestine was prepared by the method of Kakemi, *et al.*[14] After drying the mucosa at 100°, 2% (dry weight) homogenates were prepared in a teflon homogenizer with the various pH buffer solutions used in this experiment. The equilibrium dialysis method employed by Klotz[15] was adopted to estimate the binding. Ampicillin and Amoxicillin were dissolved in the various pH buffer solutions to concentration of 8×10^{-5} M. Two ml of the mucosal homogenate inside a dialysis bag (Visking Co. 8/32) were equilibrated for 24 hr at 37° with 5 ml of the drug solution. Percentage of binding was calculated from the difference of drug concentration in the outer fluid in the presence of mucosa from that in the absence of mucosa.

Results and Discussion

Membrane Permeability and Physicochemical Properties

The results of the experiments on membrane permeability using everted intestines from various segments of the rat digestive tract indicated that there was no significant difference in the membrane permeability through these various segments among ampicillin, amoxicillin and aminobenzylpenicilloic acid (Fig. 1). From this result, it is assumed that aminobenzylpenicilloic acid can be absorbed from the small intestine.

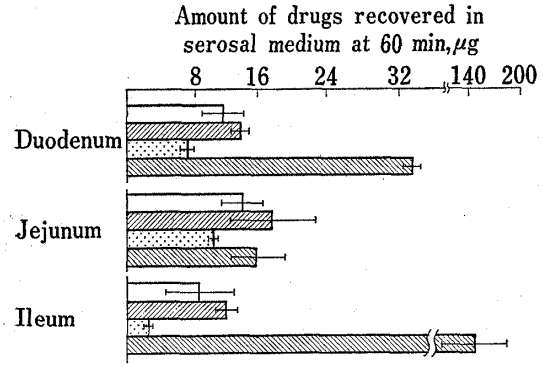

Fig. 1. Permeation of Drugs through the Everted Rat Intestine

Results are expressed as the mean ± S.E. of at least four animals.
□: ampicillin
▨: amoxicillin
▦: aminobenzylpenicilloic acid
▩: pivampicillin

The membrane permeability of pivampicillin through the duodenum and ileum was significantly higher than other drugs tested. On the basis of these results, the fact[5b] that pivampicillin has achieved high levels in the body fluids can be explained. And this findings, the higher levels of pivampicillin, can also be explained on the basis of the physicochemical properties of these drugs which were investigated. As shown in Fig. 2 and Table I, the apparent partition coefficient of pivampicillin was much greater than that of other antibiotics, and the dissolution rate in water of pivampicillin released from the capsule preparation was higher than that of other antibiotics.

12) R.R. Levine and E.W. Pelikan, *J. Pharmacol. Exptl. Ther.*, **131**, 319 (1961).
13) W.T. Agar, F.J.R. Hiro, and G.S. Sidhu, *Biochim. Biophys. Acta*, **14**, 80 (1954).
14) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967).
15) I.M. Klotz, F.M. Walker, and R.B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).

However it was difficult to explain the fact[5c] that amoxicillin has achieved higher levels in the body fluids than ampicillin only on the basis of the lipid solubility, dissolution rate in water and *in vitro* membrane permeability, since, unlike pivampicillin, the partition coefficient of amoxicillin was low, the dissolution rate of amoxicillin released from the capsule preparation and the tablet preparation containing only amoxicillin in water and hydrochloric acid solution was much lower than that of ampicillin (Fig. 3) and the membrane permeability of amoxicillin, was, as shown in the experimental result in Fig. 1, not significantly higher than that of ampicillin. For this reason and in order to obtain a new finding on the mechanism of membrane permeation of these amphoteric compounds, the authors studied the behavior of ampicillin and amoxicillin in the digestive tract such as *in vivo* membrane permeation and *in vitro* membrane adsorption.

Table I. Apparent Partition Coefficient for Ampicillin and Its Derivatives at 37°

Compound	Organic phase	Partition coefficient at pH		
		5.0	6.4	7.4
Ampicillin	butanol	0.18	0.24	0.40
	chloroform	0.02	0.02	0.03
Amoxicillin	butanol	0.09	0.15	0.19
	chloroform	0.02	0.01	0.01
Pivampicillin	butanol	18.05	28.27	8.51
	chloroform	69.20	26.60	5.80
AB-PA	butanol	0.15	0.11	0.08
	chloroform	0.02	0.04	0.03

AB-PA: aminobenzylpenicilloic acid
apparent partition coefficient $= (C_i - C_e)/C_e$
C_i: initial concn. of drug in the aqueous layer
C_e: concn. of drug in the aqueous layer at equilibrium

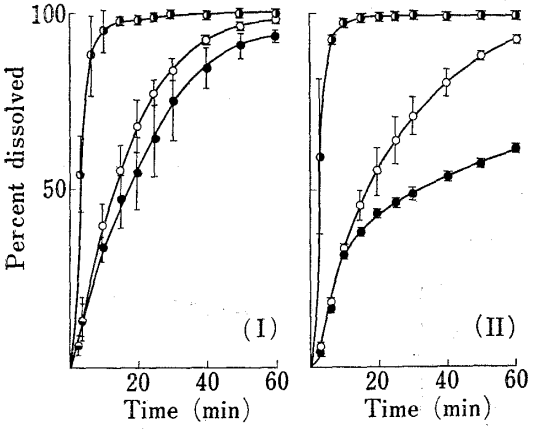

Fig. 2. Dissolution Curves of Ampicillin (○), Amoxicillin (●) and Pivampicillin (◑) from Capsules in Redistilled Water at 37°

(I) : NF XIII rotating basket method
(II): beaker method
Results are expressed as the mean±S.D. of four experiments.

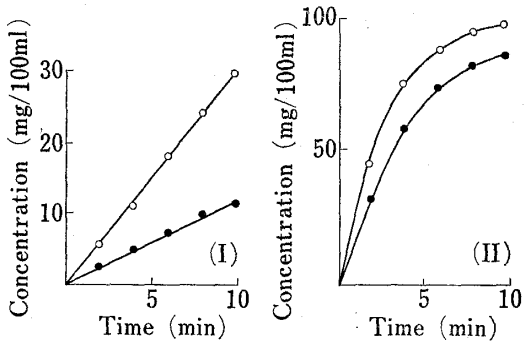

Fig. 3. Dissolution Curves of Ampicillin (○) and Amoxicillin (●) from Compressed Disk in Redistilled Water and pH 1.2 Hydrochloric acid at 37°

(I) : in redistilled water
(II): in hydrochloric acid
Results are expressed as the mean of two experiments.

Since it was considered that the influence of blood stream had to be taken into account, absorption of these antibiotics was studied by determining a remaining ratio with the loop method (Fig. 4). As a result it was found that the remaining ratio of ampicillin was significantly higher than that of amoxicillin when the test solutions of pH 6.4 and pH 7.4 were used ($p<0.01$). In addition, as shown in Fig. 5, showing the relationship between the remaining

ratio and partition coefficient of amoxicillin and ampicillin at each of the pH's, it was found that absorption of amoxicillin was better than that of ampicillin despite the fact that the partition coefficient of amoxicillin was lower than that of ampicillin at each pH.

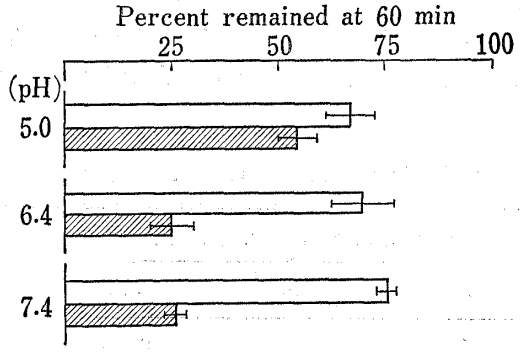

Fig. 4. Absorption of Ampicillin and Amoxicillin from the Rat Intestinal Loops at Various pH's

Duodenum (7 cm) was used as loops.
Results are expressed as the mean±S.E. of four animals.
□: ampicillin ▨: amoxicillin

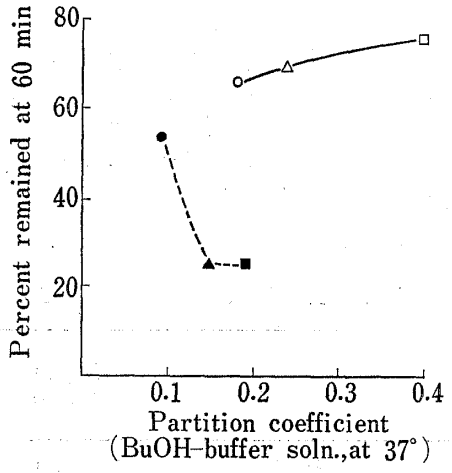

Fig. 5. Relationship between Absorption and Partition Coefficients of Ampicillin and Amoxicillin

Percent remained was obtained in the loop (7 cm) of rat duodenum.
——: ampicillin ------: amoxicillin
○●: pH 5.0, △▲: pH 6.4, □■: pH 7.4
apparent partition coefficient $= \frac{C_i - C_e}{C_e}$
C_i: initial concn. of drug in the aqueous layer
C_e: concn. of drug in the aqueous layer at equilibrium

Accumulation and Binding of Ampicillin and Amoxicillin

Accumulation of ampicillin and amoxicillin was studied using everted intestinal slices (Fig. 6). As shown in Fig. 6, it was found that accumulation of amoxicillin by the membrane was significantly greater than that of ampicillin ($p<0.01$).

In another experiment, pH profiles of the binding of ampicillin and amoxicillin to the mucosa was investigated (Fig. 7). In this experiment also, it was found that the binding of

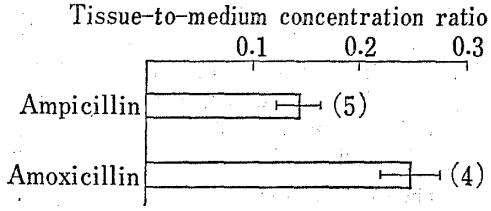

Fig. 6. Accumulation of Ampicillin and Amoxicillin by the Everted Rat Intestinal Slices at pH 7.4

Results are expressed as the mean±S.E. of four and five animals respectively.

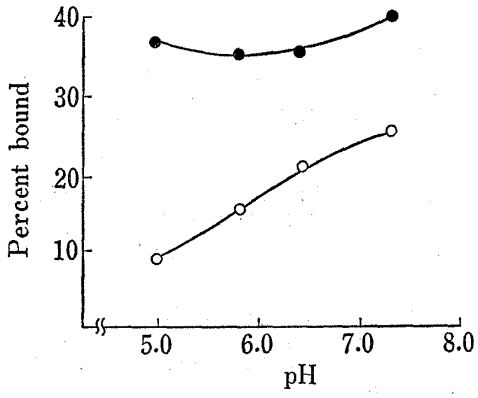

Fig. 7. pH Profiles of Binding of Ampicillin and Amoxicillin to the Rat Intestinal Mucosa

equilibrium dialysis for 24 hr at 37°
Results are expressed as the mean of two experiments.
—○—: ampicillin —●—: amoxicillin

amoxicillin onto the mucosa was significantly greater than that of ampicillin at the various pH media tested.

These findings are assumed to be evidence for the concept that the interaction as expressed by the *in vitro* accumulation and binding to mucosa are influencing factors in the absorption of ampicillin and amoxicillin.

On the basis of these results, further binding studies of ampicillin, amoxicillin and other β-lactam antibiotics to the various membrane components of the rat intestinal mucosa and the relationships between the binding to the membrane components and the *in vivo* absorption properties would be of interest and of importance.

Transport Mechanisms of Ampicillin and Amoxicillin across Small Intestine

Figure 8 shows the results for the effect of drug concentrations in the mucosal fluids on the permeation of ampicillin and amoxicillin through the everted rat intestinal sacs. The permeation of these drugs to the serosal side was dependent on the mucosal drug concentrations within the concentration range tested.

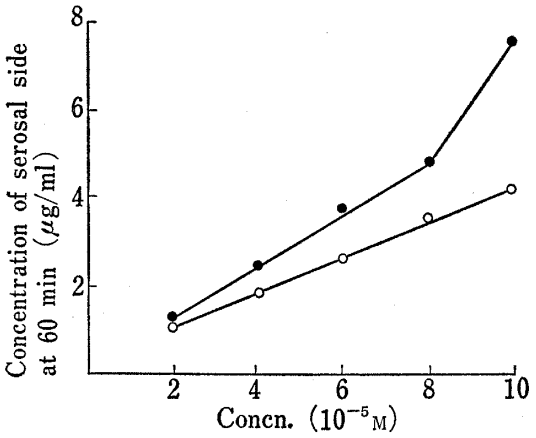

Fig. 8. Effects of Drug Concentrations on the Permeation of Ampicillin and Amoxicillin through the Everted Rat Intestinal Sacs

Results are expressed as the mean of two animals.
—○—: ampicillin —●—: amoxicillin

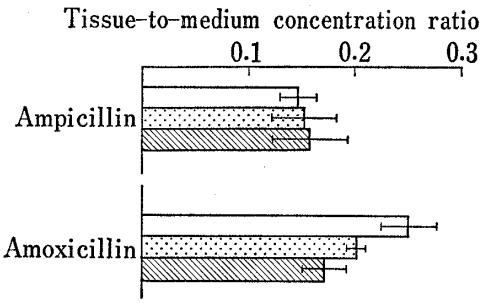

Fig. 9. Effects of Metabolic Inhibitors on the Accumulation of Ampicillin and Amoxicillin by the Everted Rat Intestinal Slices at pH 7.4

Results are expressed as the mean±S.E. of four and five animals.
□: control ▨: NaN_3 (30 mM),
▨: DNP (0.5 mM)

In the experiment to determine the effect of metabolic inhibitors on the accumulation of these drugs by the rat everted intestinal slices, the tissue-to-medium concentration ratio in the presence of DNP or NaN_3 was found no to be significantly different from that of the control according to the Student's t test (Fig. 9). In addition, in the control experiment, the tissue-to-medium concentration ratios were much smaller than 1.

Furthermore, in another experiment using the everted sac, uphill transport mechanisms of ampicillin and amoxicillin were not observed, as shown in Table II.

Table II. Final Serosal-to-Mucosal Concentration Ratio of Ampicillin and Amoxicillin across the Everted Rat Intestinal Sacs

Compound	S/M±S.D.
Ampicillin	0.87±0.04
Amoxicillin	0.87±0.05

Results are expressed as the mean ± S.E. of five animals.

Studies on the transport mechanism of β-lactam antibiotics (penicillins and cephalosporins) in the everted rat small intestine have recently been reported by Penzotti and Poole,[4] but amoxicillin was not examined and relatively higher antibiotic concentrations were employed in this experiment.

In the present study, the authors used relatively lower concentrations of ampicillin and amoxicillin. From these experiments, it is concluded that ampicillin and amoxicillin are transported across the everted rat small intestine by passive diffusion, which is in agreement with the results of Penzotti and Poole.[4]