

Intestinal Absorption of Several β -Lactam Antibiotics. II.¹⁾ Absorption Characteristics of Amino-penicillins and Amino-cephalosporins

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In order to investigate the absorption characteristics of cephalexin, cephradine, and amoxicillin, which are well absorbed from the gastrointestinal tract, the mechanisms of intestinal absorption of these antibiotics in rats were examined using the recirculating perfusion technique, the loop method, and the everted gut sac technique. Cephradine was shown to be actively transported. Other antibiotics appeared to diffuse across the intestine down a concentration gradient. The accumulations of these drugs, in the intestinal tissue, especially cephalexin and cephradine, were particularly marked, and the accumulation of each of these three drugs was significantly inhibited at low temperature. These observations provide an experimental basis for the known rapid absorption of these antibiotics.

Keywords—intestinal absorption mechanisms; β -lactam antibiotics; cephalexin; cephradine; amoxicillin; ampicillin; active transport; tissue accumulation

It is well known that amino-penicillins and amino-cephalosporins are well absorbed from the small intestine even though they are completely ionized at all pH values and have very low lipid solubilities. Much interest has also been focused on the absorption mechanisms of these amphoteric β -lactam antibiotics across the intestinal membrane.^{1a,3,4)}

In connection with the absorption of amino-penicillins, Dixon and Mizen^{3a)} reported that cyclacillin could be actively transported across everted rat intestine but that ampicillin and amoxicillin were passively transported. The authors also reported^{1a)} similar results for ampicillin and amoxicillin suggested that a difference of affinity for the intestinal tissue may account for the difference in absorption rates of these penicillins.

However, there have been only a few reports on the transport mechanisms of amino-cephalosporins. The study reported here was undertaken to investigate the absorption properties of amino-cephalosporins such as cephalexin and cephradine in comparison with those of amino-penicillins, using the new fluorometric determination methods⁵⁾ reported previously. The involvement of specialized transport mechanisms was also examined at low drug concentrations by the everted gut sac technique.

- 1) a) Part I: K. Miyazaki, O. Ogino, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **25**, 246 (1977); b) A part of this work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977.
- 2) Location: *Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan.*
- 3) a) C. Dixon and L.W. Mizen, *J. Physiol.*, **269**, 549 (1977); b) A. Tuji, E. Nakashima, I. Kagami, T. Asano, R. Nakashima, and T. Yamana, *J. Pharm. Pharmacol.*, **30**, 508 (1978); A. Tuji, E. Nakashima, E. Kagami, N. Honjo, and T. Yamana, *ibid.*, **29**, 707 (1977); c) T. Kimura, H. Endo, M. Yoshikawa, S. Muranishi, and H. Sezaki, *J. Pharm. Dyn.*, **1**, 262 (1978).
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Experimental

Materials and Reagents—The chemical structure of β -lactam antibiotics covered in this report are presented in Chart 1. The monohydrate of cephalixin (potency: 935 $\mu\text{g}/\text{mg}$), dihydrate of cephadrine (potency: 950 $\mu\text{g}/\text{mg}$), and trihydrate of amoxicillin (potency: 856 $\mu\text{g}/\text{mg}$) were kindly supplied by Shionogi and Co., Osaka, Japan, Sankyo Co., Tokyo, Japan, and Kyowa Hakko Kogyo Co., Tokyo, Japan, respectively. Anhydrous ampicillin (potency: 1025 $\mu\text{g}/\text{mg}$) and anhydrous cyclacillin (potency: 1049 $\mu\text{g}/\text{mg}$) were kindly supplied by Takeda Chemical Industries, Osaka, Japan. All the reagents were of special grade, and were prepared with redistilled water. Quinine sulfate solution, the standard solution used in these experiments, was prepared as described previously.^{5a)}

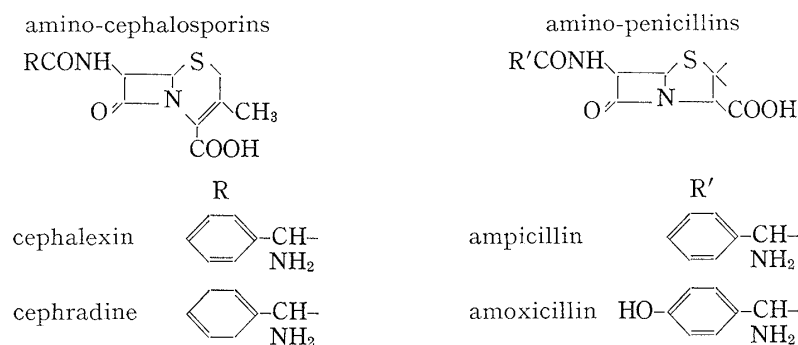


Chart 1. Structures of β -Lactam Antibiotics tested

Apparatus for Fluorometric Determination—Fluorescence intensity was measured with a Hitachi 203 spectrofluorometer equipped with a xenon lamp.

Procedure for Absorption Experiments—Wistar male rats (150–250 g) were anesthetized with ether and sodium pentobarbital (2 mg/100 g). Whole small intestine, loops, and everted sacs were used in the absorption experiments.

a) *In Situ* Recirculating Perfusion Experiment: The technique of Kakemi *et al.*⁶⁾ was employed. The drugs were dissolved in isotonic phosphate buffer with a pH of 6.4 (100 $\mu\text{g}/\text{ml}$). The bile duct was ligated in all experiments. Before experiments, the intestine was perfused with 50 ml of normal saline, maintained at 37°, and then with 30 ml of drug-free buffer solution to wash and bufferize the intestine. One-half ml of the sample solution was pipetted in at 10, 15, 20, 30, 40, 50, and 60 min after the start of recirculation. The perfusion solution (50 ml) was recirculated at a rate of 6 ml/min.

b) Loop Experiment: The drugs were dissolved in isotonic phosphate buffer, pH 6.4 or 7.4 (50 $\mu\text{g}/\text{ml}$). A single loop from the upper small intestine was prepared 7 cm from the pylorus according to the method of Levine Pelikan.⁷⁾ The bile duct was ligated. After washing the loop gently with 10 ml of the drug-free buffer solution used in this experiment, 1 ml of drug solution was injected into the loop. At selected intervals before the animal was sacrificed, the loop was removed and the contents were emptied into a 25 ml volumetric flask. The mucosal side of the loop was thoroughly rinsed with redistilled water to give a volume of 25 ml.

c) Everted Sac Experiment: A large section of small intestine, starting below the pyloric sphincter was removed from the rat, and rinsed with iced saline. The first 15 cm segment was discarded. The next 30 cm segment was everted, and divided into three 10 cm segments. Various concentrations of drug solutions were prepared with modified Ringer's solution⁸⁾ with a pH of 7.4. The serosal initial volume was 1.0 ml. These three sacs from different animals were each placed in a 200 ml Erlenmeyer flask containing 100 ml of the same drug solution (mucosal side), previously equilibrated at 37° or 27°, and each flask was continually gassed with 5% carbon dioxide in oxygen. At the end of the incubation period (1 hr), each sac was emptied into a 10 ml volumetric flask and 0.5 ml of serosal fluid was diluted with redistilled water. In the determination of tissue concentration, 4.5 ml of homogenate was prepared in a Teflon homogenizer with redistilled water.

Furthermore, in other experiments, 2,4-dinitrophenol (DNP), sodium azide (NaN_3), and ouabain were added to both the serosal and mucosal sides to estimate the effects of metabolic inhibitors on the transport of these drugs was also examined.

Drug Accumulation on the Everted Intestinal Slices—Accumulation of amino-cephalosporins on the digestive tract membrane was compared with that of amino-penicillins by preparing slices of 0.5 cm thickness

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7) R.R. Levine and E.W. Pelikan, *J. Pharmacol. Exptl. Ther.*, **131**, 319 (1961).

8) S.G. Schulz, R.E. Fuisz, and P.F. Curran, *J. Gen. Physiol.*, **49**, 849 (1966).

from the everted intestine of rats (proximal jejunum, 0.5 g wet weight).⁹⁾ The slices were placed in 100 ml Erlenmeyer flasks with 50 ml of modified Ringer's solution containing 8×10^{-5} M drug concentration, and the flask was gassed with 5% carbon dioxide in oxygen 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 carefully with filter paper to remove adhering moisture. Five ml of homogenate was then prepared in a Teflon homogenizer with redistilled water.

Analytical Method—All the antibiotics used in this experiment were measured by the fluorometric determination methods⁵⁾ reported previously.

Results

Comparative Study of Intestinal Absorption

Figure 1 shows the results for ampicillin, cephalixin, and cephradine when these drugs were recirculated through the small intestine. Cephalixin and cephradine disappeared rapidly from the intestine, and steady states were observed at about 20 min.

The disappearance from the intestinal lumen of cephalixin, cephradine, ampicillin, and amoxicillin was also examined by the loop method. It was found that the ratios of amino-cephalosporins remaining were significantly lower than those of amino-penicillins. Of the two amino-penicillins, the disappearance of amoxicillin was greater than that of ampicillin (Fig. 2). Similar results were also obtained when drug solutions of pH 7.4 were used.

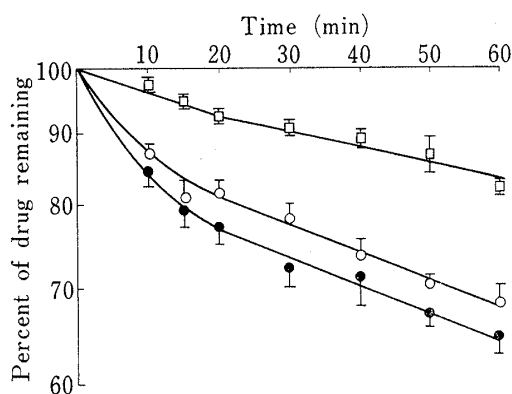


Fig. 1. Disappearance Profiles of Cephalixin (○), Cephradine (●), and Ampicillin (□) from Rat Intestinal Lumens at pH 6.4 as a Function of Time

Results are expressed as the means \pm S.E. of at least three animals.

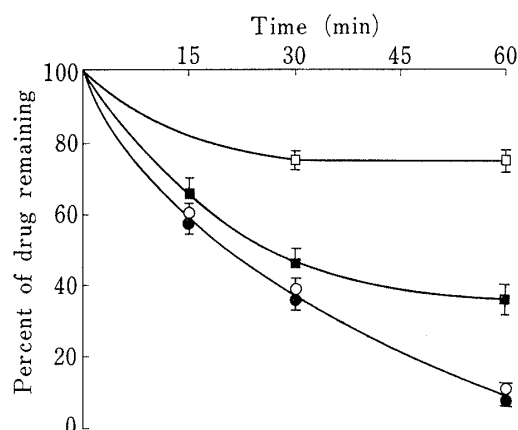


Fig. 2. Disappearance Profiles of Cephalixin (○), Cephradine (●), Amoxicillin (■), and Ampicillin (□) from Rat Intestinal Loops at pH 7.4 as a Functions of Time

Results are expressed as the means \pm S.E. of at least three animals.

Figure 3 shows the amounts of these drugs accumulated in 30 min on the everted intestinal slices. It was found that the wall tissue concentrations of amino-cephalosporins were significantly higher than those of amino-penicillins. These findings appear to support the view that specialized transport mechanisms participate in the membrane transport of cephalixin and cephradine. On the basis of these results, further studies of cephalixin, cephradine, and amoxicillin were carried out by the everted sac technique.

Transport Mechanisms of Cephalixin, Cephradine, and Amoxicillin across the Small Intestine

Figure 4 shows the effect of drug concentration in the mucosal fluids on the transport of cephalixin and cephradine across everted rat intestinal sacs. A study of the permeation of cephalixin to the serosal side showed that there was a linear increase in the rate of transport

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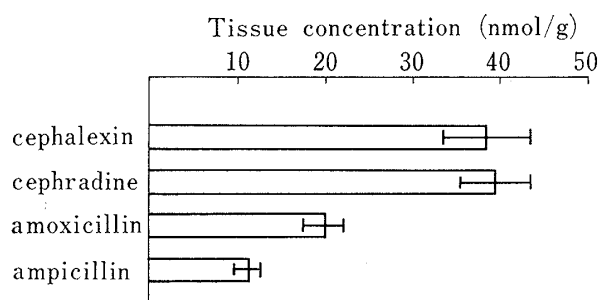


Fig. 3. Tissue Accumulation of Cephalixin, Cephradine, Amoxicillin, and Ampicillin in Everted Rat Intestinal Slices at pH 7.4

The medium concentration was 8×10^{-5} M. Results are expressed as the means \pm S.E. of at least five experiments.

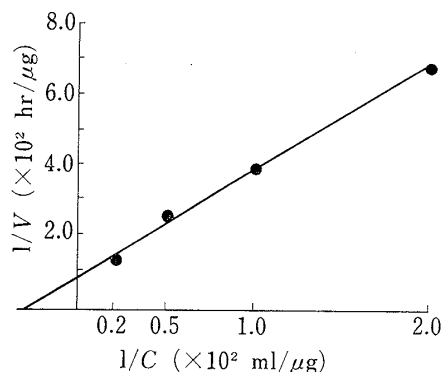


Fig. 5. Lineweaver-Burk Plots of Cephradine Transfer across Everted Rat Intestinal Sacs at pH 7.4

Results are expressed as the means of three experiments.

$$V_{\max} = 0.318 \mu\text{mol/hr} \quad K_m = 0.924 \mu\text{mol/ml}$$

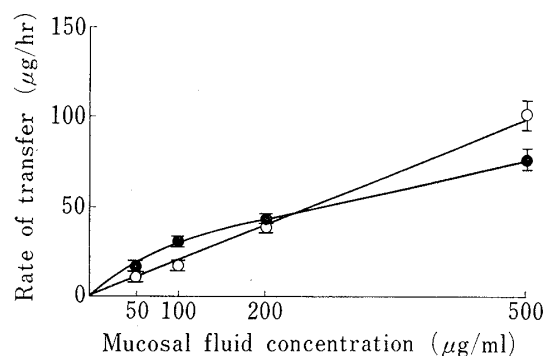


Fig. 4. Effects of Drug Concentration on the Transport of Cephalixin (\circ) and Cephradine (\bullet) across Everted Rat Intestinal Sacs at pH 7.4

Results are expressed as the means \pm S.E. of three experiments.

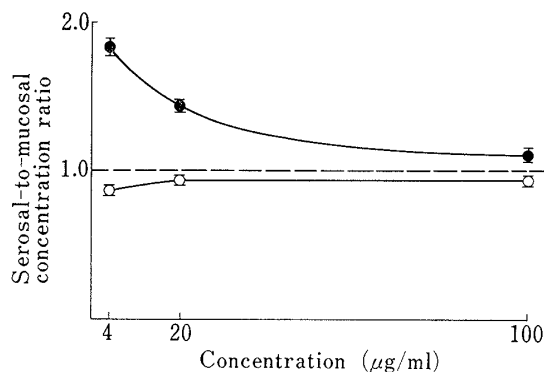


Fig. 6. Final Serosal-to-Mucosal Concentration Ratios of Cephalixin (\circ) and Cephradine (\bullet) across Everted Rat Intestinal Sacs at pH 7.4

The initial serosal-to-mucosal concentration ratio was 1.0.

Results are expressed as the means \pm S.E. of six experiments.

as the mucosal concentration of cephalixin increased. However, the permeation of cephradine was found to be saturable (Fig. 4). Figure 5 shows that the transport data for cephradine can be treated in a manner similar to that for enzyme kinetics.

As shown in Fig. 6, furthermore, at low and equal drug concentrations in serosal and mucosal solutions there was a typical difference in the serosal-to-mucosal concentration ratio between cephalixin and cephradine. Thus, it appears that cephradine was concentrated against a gradient at relatively low mucosal concentrations.

In order to study the effects of metabolic inhibitors and of temperature on the transport across the membrane and accumulation in the intestinal tissue of cephalixin, cephradine, and amoxicillin, further studies using everted sacs were carried out at pH 7.4, using an initial concentration of 5 μ g/ml in the mucosal and serosal fluids.

The results for cephradine are presented in Fig. 7. The transfer of cephradine was significantly inhibited by DNP and at 27° ($p < 0.001$). The amount of cephradine accumulated on the intestinal tissue was also reduced by DNP and at low temperature.

As shown in Fig. 8, there were also significant decreases in the serosal fluid concentration and tissue concentration of cephalixin at low temperature. It was found, furthermore, that the accumulation of cephalixin was inhibited to a greater extent by DNP than that of

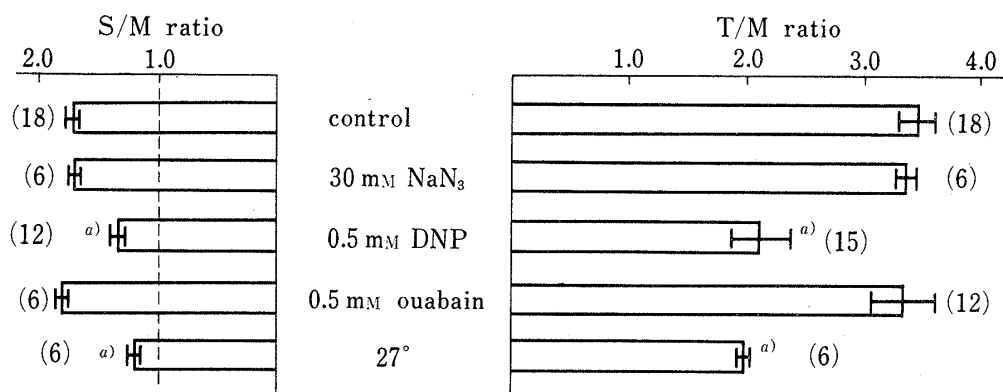


Fig. 7. Effects of Metabolic Inhibitors and Temperature on the Transport and Accumulation of Cephadrine by Everted Rat Intestinal Sacs at pH 7.4

The initial concentration in mucosal or serosal fluids was 5 µg/ml.
 S/M ratio: final serosal-to-mucosal fluids concentration ratio.
 T/M ratio: final tissue (µg/g)-to-mucosal fluid concentration ratio.
 Results are expressed as the means ± S.E.
 Numbers in parentheses represent the numbers of experiments.
 Statistically significant differences from the control: a) $p < 0.001$.
 All the experiments were carried out at 37° except for the experiment at 27°.

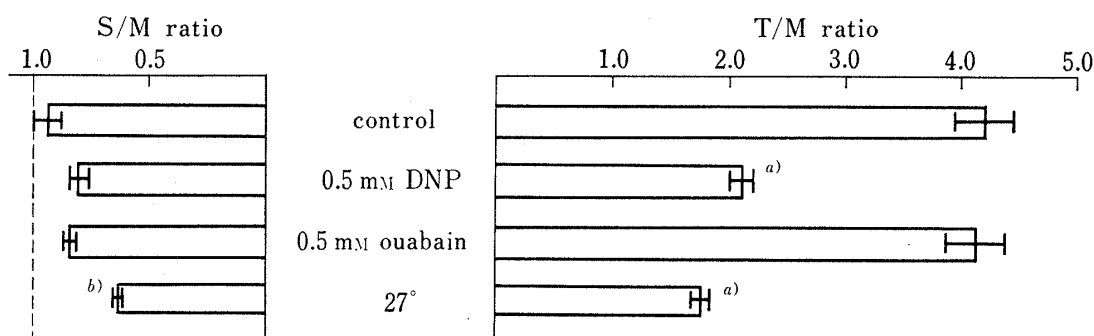


Fig. 8. Effects of Metabolic Inhibitors and Temperature on the Transport and Accumulation of Cephalexin by Everted Rat Intestinal Sacs at pH 7.4

The initial concentration on mucosal or serosal fluids was 5 µg/ml.
 S/M ratio: final serosal-to-mucosal fluids concentration ratio.
 T/M ratio: final tissue (µg/g)-to-mucosal fluid concentration ratio.
 Results are expressed as the means ± S.E. of six experiments.
 Statistically significant differences from the control: a) $p < 0.001$, b) $p < 0.005$.
 All the experiments were carried out at 37° except for the experiment at 27°.

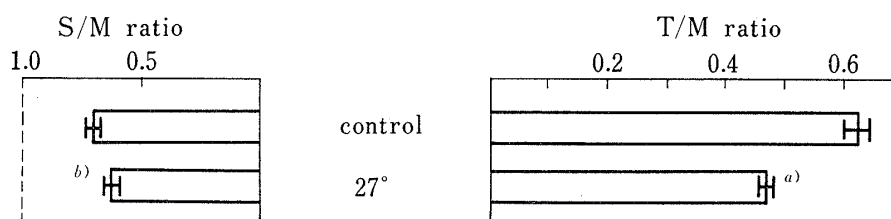


Fig. 9. Effects of Temperature on the Transport and Accumulation of Amoxicillin by Everted Rat Intestinal Sacs at pH 7.4

The initial concentration in mucosal or serosal fluids was 5 µg/ml.
 S/M ratio: final serosal-to-mucosal fluids concentration ratio.
 T/M ratio: final tissue (µg/g)-to-mucosal fluid concentration ratio.
 Results are expressed as the means ± S.E. of three (control) and six experiments.
 Statistically significant differences from the control: a) $p < 0.025$, b) $p < 0.05$.
 The control experiment was carried out at 37°.

cephradine. However, no inhibition was found with ouabain. It can therefore be assumed that Na-K ATP_{ase} is probably not involved in the transport processes of cephalixin and cephradine.

Figure 9 shows the results for amoxicillin. Although the amount of amoxicillin accumulated in the intestinal tissue was significantly inhibited at low temperature ($p < 0.025$), the tissue-to-medium concentration ratio was far lower than those of cephalixin and cephradine.

Discussion

It has been reported^{5c,10)} that more than 90—95% of these amino-cephalosporins can be absorbed from the gastrointestinal tract in 6 hr. In the present study, the rates of disappearance of cephalixin, cephradine, and amoxicillin from the small intestinal lumen were significantly higher than that of ampicillin, and the rates of disappearance of amino-cephalosporins were greater than that of amoxicillin. From the results shown in Fig. 3, it appears that differences in affinity for the intestinal tissue may provide an explanation for the differences in the absorption rates of these drugs.

Involvement of specialized transport mechanisms in the absorption of these amino-cephalosporins was examined by the everted sac technique. Cephradine was shown to concentrate against a gradient; its transfer and accumulation required metabolic energy. However, even at low and equal concentrations, the ratio of serosal-to-mucosal concentrations remained unity for cephalixin. Although the tissue concentration of cephradine was of the same order of magnitude as that of cephalixin (Figs. 3, 4, and 5), the concentration of cephradine in serosal fluids was higher than that of cephalixin (Figs. 4 and 5). Further, in the transport of cephradine, the inhibition of accumulation on the intestinal tissue was similar in extent to that of transfer in the presence of DNP. Thus, it seems reasonable to assume that a specific transfer step is a major factor in the absorption of cephradine. It is possible that a specific accumulation process from the medium to the mucosal tissue is also involved in the absorption process of cephalixin.

In order to confirm the involvement of active transport mechanisms in cephradine absorption, the effect of cyclacillin (100 $\mu\text{g}/\text{ml}$) on the transport process of cephradine (5 $\mu\text{g}/\text{ml}$) was examined by the everted sac technique. There were significant decreases in serosal fluid concentration and in wall tissue concentration of cephradine in the presence of cyclacillin.¹¹⁾

In conclusion, evidence has been presented to show that cephradine is actively transported across the everted rat intestine. This was not the case for cephalixin or amoxicillin. However it seems possible that a specific interaction requiring metabolic energy for the binding to the intestinal mucosa influences the rapid absorption of cephalixin and amoxicillin. Further studies are required to investigate the properties of these specific interactions.

10) R. Aikawa, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **24**, 2350 (1976).

11) K. Umeniwa, K. Miyazaki, and T. Arita, unpublished data.