707

Effect of dose-concentration on the absorption of amoxicillin and ampicillin from the rat intestine

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In spite of being completely ionized structures at all pH values and also having very low lipid solubility, amino-penicillins such as amoxicillin and ampicillin are well absorbed from the alimentary tract to produce a therapeutic effect (Bodey & Nance, 1972; Gordon, Regamey & Kirby, 1972; Sutherland, Croydon & Rolinson, 1972; Philipson, Sabath & Rosner, 1975). To clarify the absorption mechanism of the amino-penicillins, we have studied their membrane permeability across the rat small intestine, both by the *in situ* loop and recirculating perfusion techniques.

Male Wistar rats (150–250 g) were fasted for 20 h before being used. They were anaesthetized with urethane (1·5 g kg^{-1}, i.p.), a midline incision was made to expose the small intestine, and the bile duct was ligated. The intestinal lumen was gently washed with the isotonic phosphate buffer. For the loop method, the small intestine was ligated into four portions of 5 cm length (see Fig. 1 for explanation). One ml of 20 μg ml^{-1} or 2 mg ml^{-1} solution of amoxicillin trihydrate or ampicillin anhydrate prepared in pH 7·4 isotonic phosphate buffer was injected directly into each doubly ligated segment and left for 1 h.

The recirculating perfusion method used was essentially according to Schanker, Tacco & others (1958), and 9 ml of pH 7·0 isotonic phosphate buffer containing amino-penicillin was continuously recirculated at the rate of 2 ml min^{-1} through a 30 cm portion from the pylorus for 2 h; a constant pH was maintained by a pH-stat (Radiometer, Copenhagen).

At the end of the experiment, the remaining antibiotic solution was collected and the intestine was washed thoroughly with the isotonic buffer. The solutions were combined to make the desired volume. The extents of apparent absorption were calculated from the sum of the amounts of the residual amino-penicillin and the degradation product, the penicilloic acid, assayed by fluorometric determination (Miyazaki, Ogino & others, 1975; 1977b). The percent absorptions from the loop for amoxicillin and ampicillin are given in Fig. 1, along with the percent of the degradation products. The degradation of two antibiotics occured to a similar extent.

The absorption of amoxicillin was significantly higher than that of ampicillin at each intestinal segment (*P* < 0·05). These results are consistent with the fact that amoxicillin gives distinctly better blood concentrations than ampicillin after intraduodenal administration to rats (Miyazaki & others, 1977b). At a low concentra-
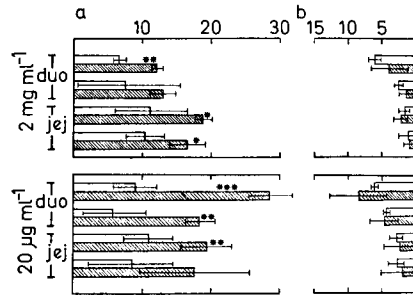
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Fig. 1. The % of a—absorption and b—degradation obtained from the rat intestinal loop of amoxicillin (hatched columns) and ampicillin (open columns). All studies were over 1 h. Each dose was dissolved in pH 7·4 isotonic phosphate buffer and injected in a volume of 1 ml into a 5 cm intestinal loop. In each experiment, two loops were prepared. The first loop was made at 2 cm from the pylorus, with 1 cm of intestine separating the consecutive loop in duodenum. For the study in jejunum, the loops were prepared at 15 cm from the pylorus. The points represent the mean absorption in 2 to 5 rats with standard error shown as a bar. The significances between amoxicillin and ampicillin are indicated as follows: *P* < 0·05, ** *P* < 0·01 and *** *P* < 0·001, duo—duodenum, jej—jejunum.

tion (20 μg ml^{-1}), amoxicillin was well absorbed from the upper part of the duodenum (*P* < 0·001).

We also observed a significant effect (*P* < 0·001) of concentration on the absorption of amoxicillin (Fig. 1). To confirm this phenomenon, kinetic experiments using the *in situ* recirculating perfusion technique were made at pH 7·0 with various concentrations ranging from 5 to 2100 μg ml^{-1} of amoxicillin. The results of absorption and tissue accumulation are illustrated in Fig. 2.

Above 100 μg ml^{-1}, as the absorption rates are constant, there is a proportional increase in the amount absorbed. For example, at concentrations of 210 and 2100 μg ml^{-1}, these values (mean ± s.e.) were 10·4 ± 1·8 and 11·1 ± 1·8%, respectively. The results indicate that at these higher concentrations the passive diffusion is the dominant absorption mechanism. But in the region below 100 μg ml^{-1}, the absorption increased with decrease in the initial drug concentration (Fig. 2). Typical results repeated for at least three animals were 43·4 ± 5·4 and 30·3 ± 11·1%, for the initial drug concentration of 21·0 and 42·0 μg ml^{-1}, respectively. The accumulation of amoxicillin in the small intestine, which was determined from the gut homogenate, was very low (1·9 ± 1·5, 1·6 ± 0·9 and 1·0 ± 0·1% respectively at 21·0, 210 and 2100 μg ml^{-1}) compared with the apparent absorption. It is expected that the amount of the drug
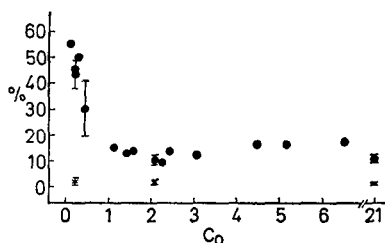
FIG. 2. Plots of the % absorption (●) and tissue accumulation (×) against initial concentration of amoxicillin, C_0 (μg ml^{-1} × 10^{-2}) at pH 7·0 from the rat small intestine by the *in situ* recirculating perfusion technique. The perfusion solution (9 ml) was recirculated at a rate of 2 ml min^{-1}. The small intestine was a 30 cm length from the pylorus. Vertical bars represent the standard error.

removed from the intestine almost completely transfers into the blood. The relation between amount absorbed and the initial drug concentration suggests that the absorption of amoxicillin is likely to follow the simultaneous kinetics of a simple diffusion process predominant at high concentrations and a Michaelis-Menten process which can only be seen at low concen-

trations. Recently, Miyazaki, Ogino & others (1977a) have observed, by the method of the isolated everted rat intestine, that there was no uphill transport of amoxicillin. These findings together with site specificity in absorption as observed in the *in situ* loop method suggest that facilitated diffusion and not active transport is involved in the absorption of low dose of amoxicillin. The present observations, however, do not demonstrate the nature of the transport mechanism, and are nothing more than an indication of a saturable rate-limiting step in the absorption process of amoxicillin.

With the *in situ* recirculating absorption experiment for ampicillin at low concentration, the extents of the absorption were relatively low and variable in each animal. For example, at 17 μg ml^{-1}, the percent absorption was 9·6 $\pm$ 8·1 %. Owing to the unexpectedly low percentage of absorption and its large variance, we could not judge whether for ampicillin there was an absorption phenomenon similar to that for amoxicillin. The large degradation seen with the perfusion technique compared with the *in situ* loop method occured with both antibiotics, and their degradation percentages were of the same order of magnitude; 28·7 $\pm$ 8·6 % at concentration of 17 μg ml^{-1} for ampicillin and 29·1 $\pm$ 21·8 % at concentration of 21 μg ml^{-1} for amoxicillin.

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The mechanism of the release of prostaglandin-like activity from guinea-pig isolated ileum

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Botting & Salzmann (1974) demonstrated that PGE$_2$ was released from guinea-pig isolated ileum during field stimulation, but that the release was only pronounced at frequencies of 10 Hz and above. Since Hughes (personal communication) had shown that noradrenaline release from intramural nerves of field-stimulated guinea-pig ileum was only easily detectable at similar frequencies it was possible that the synthesis of prostaglandin was a consequence of neuronal release of noradrenaline.

This suggestion was investigated using strips of guinea-pig terminal ileum suspended in an organ bath (volume 5 ml). The bath fluid and other experimental conditions were as described previously (Botting & Salzmann, 1974). The tissue was left for 90 min during which the fluid was changed every 10 min. The bath fluid was then collected and replaced with fresh solution every 15 min. During alternate periods field stimulation was applied by silver electrodes connected to an S.R.I.