ACKNOWLEDGMENTS AND ADDRESSES

Received March 27, 1975, from the *Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712, and the ‡Division of Medicinal Chemistry and Natural Products, College of Pharmacy, University of Iowa, Iowa City, IA 52242

Accepted for publication June 11, 1975.

Presented at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, April 1975.

Supported in part by Grant NS-12259, National Institute of Neurological Diseases and Stroke, National Institutes of Health.

The authors are grateful to Dr. Robert J. Borgman, School of Pharmacy, West Virginia University, Morgantown, W.Va., for providing the N-methyl-3,4-dimethoxyamphetamine and N,N-dimethyl-3,4-dimethoxyamphetamine samples.

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Intestinal Secretion of Erythromycin Base

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Abstract □ Erythromycin fluxes into rabbit midjejunal segments were studied. When erythromycin was infused into the jugular vein of anesthetized rabbits, the antibiotic was secreted into the segments at a rate of 0.0136 ± 0.0023 mg/min. Preloading of the segments with five and 20 times the plasma concentration did not diminish this secretion. Protein binding of the antibiotic within the lumen could not explain this secretion, since both ultrafiltration and chromatography of luminal solutions indicated that the biological activity was free erythromycin. Moreover, the transmural potential across the intestinal mucosa is not likely to be the principal driving force, since greater than 80 mv would be required to sustain the observed secretion against an imposed 20-fold concentration difference between blood and lumen. The best explanation for the intestinal secretion of erythromycin appears to be an active transport pathway capable of concentrating erythromycin in the lumen. It is not clear what endogenous substances are transported by this pathway.

Keyphrases □ Erythromycin—intestinal secretion, rabbit midjejunal segments □ Intestinal secretion—erythromycin, rabbit midjejunal segments □ Antibiotics—erythromycin, intestinal secretion, rabbits

In humans, oral administration of erythromycin free base produces low blood levels of antibiotic. For example, a 250-mg tablet of erythromycin free base produces average peak blood levels of 0.12–0.54 μg/ml (1, 2). In comparison, similarly administered propionyl erythromycin produces peak blood levels of 1.11–1.92 μg/ml.

The lower blood levels of erythromycin base have been attributed to a number of factors including: (*a*) a deleterious effect of ingested food on absorption of the base (3), (*b*) acid degradation in the stomach (4), (*c*) the volume of distribution following absorption (5), (*d*) biliary secretion (6–8), and (*e*) a combination of low absorption rate with high renal excretion rate (9).

Preliminary studies in this laboratory confirmed the observation (10) that a fraction of intravenously administered erythromycin base appeared in the intestinal lumens of rats with extracorporeal bile fistulas. Since biliary excretion was clearly eliminated as a source of intestinal secretion, these studies suggested the direct secretion of erythromycin across the intestinal mucosa. Such a pathway of secretion might be important not only as a new route of elimination but also as an influence on the oral administration of erythromycin.

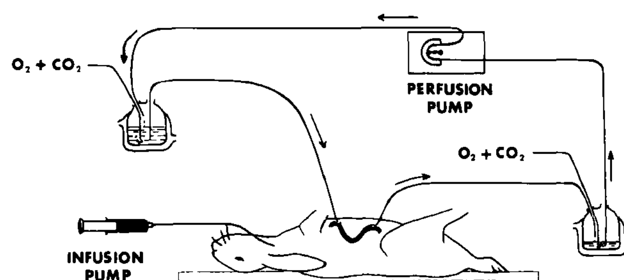

Figure 1—*Schematic representation of perfusion circuit through rabbit midjejunum.*

EXPERIMENTAL

Surgical Preparation—Male New Zealand white rabbits, 3.2–3.7 kg, were anesthetized with 50 mg/kg iv of secobarbital[1]. A 50-cm segment of midjejunum, measured 75 cm proximal to the ileocecal valve, was cannulated both proximally and distally. The segment did not include the bile duct. Krebs–Ringer bicarbonate buffer, saturated with 95% oxygen–5% carbon dioxide at 40°, was initially washed through the segment. This buffer had the following composition: Na^+, 0.143 *M*; K^+, 0.006 *M*; Ca^{+2}, 0.003 *M*; Mg^{+2}, 0.001 *M*; Cl^-, 0.128 *M*; PO_4^{-2}, 0.001 *M*; SO_4^{-2}, 0.001 *M*; and HCO_3^-, 0.025 *M*.

After rinsing with buffer, air was injected to displace excess solution. One hundred milliliters of buffer was then circulated through the segment between two water-jacketed reservoirs maintained at 40° (Fig. 1). Inflow pressure was maintained below 20 cm of water to prevent damage to the mucosa. Both reservoirs were initially bubbled with 95% oxygen–5% carbon dioxide. As the experiment progressed, the pH of the perfusate tended to increase but was maintained at a constant value of 7.4 by increasing the proportion of carbon dioxide in the gas mixture.

A polyethylene cannula in the left external jugular vein allowed vascular infusion of erythromycin. Blood samples were withdrawn through a second cannula placed in the left common carotid artery.

Perfusion Experiments—A constant concentration of erythromycin in the plasma (1 μg/ml) was maintained by a bolus injection of 3 mg into the jugular cannula followed by infusion at a rate of 5 mg/hr. The appearance of erythromycin in the intestinal segment was then followed by sampling at 20-min intervals. Carotid blood samples were taken concomitantly. The perfusate and carotid plasma were assayed by the disk-plate microbiological assay tech-

[1] Seconal, Eli Lilly and Co.

Table I—Erythromycin Flux into Intestinal Segments Preloaded with the Antibiotic

Initial Concentration in Lumen, μg/ml	n^a	Flux^b, mg/min	p^c
0	5	0.0136 ± 0.0023	<0.002
5	4	0.0138 ± 0.0036	<0.02
20	7	0.0299 ± 0.0144	<0.05

^{a} Number of animal preparations used to determine flux. ^{b} Mean ± *SE* of the mean. ^{c} Probability that fluxes did not differ from zero were determined by the Student *t* test.

nique of Davis and Stout (11). The ratio of lumen to plasma concentrations (L/P) was calculated to facilitate statistical analysis. The Student *t* test was used to determine if L/P exceeded 1, indicating net movement into the lumen.

To determine if secretion proceeded against a concentration gradient, luminal solutions were initially loaded with 5 or 20 μg/ml of erythromycin. Fluxes into the lumen with different levels of preloading were then compared. The method of calculation of fluxes was as follows. The volume of solution in the segment at each sampling time was determined by four experiments using the nonabsorbable marker ^{14}C-inulin. The product of this volume and the concentration of erythromycin in perfused buffer was the total amount of erythromycin in the lumen. The change in total luminal erythromycin with time, as determined by linear regression analysis, was taken as the flux into the lumen.

Assay differences for erythromycin in plasma and perfused buffer were evaluated by loading samples of antibiotic-free plasma and perfused buffer with 1 μg/ml of erythromycin. The L/P ratios for these inoculated controls did not differ significantly from 1.

Ultrafiltration—The binding of erythromycin to macromolecules within the intestine was studied by ultrafiltration of a sample of luminal buffer preloaded with erythromycin. The ultrafilter membrane[2] had a cutoff at a molecular weight of 30,000 with an apparent pore radius of 29 Å. The filter was saturated with *N*-methyl-^{14}C-erythromycin by passing 20 ml of 0.9% NaCl containing 3 mg/ml of radiolabeled erythromycin through the filter.

Ultrafiltrate samples (1 ml) were collected sequentially. After addition of 15 ml of scintillation cocktail[3], these samples were counted in a scintillation detector. Saturation of binding sites on the filter was judged to be complete when the erythromycin concentration in the ultrafiltrate reached a plateau of radioactivity equivalent to 3 μg/ml. The saline solution in the ultrafiltration chamber was then replaced with a 2-hr luminal solution preloaded with 3 μg/ml of radiolabeled erythromycin. This solution was then passed through the same filter, and the ultrafiltrate was collected for counting.

Chromatography—Chromatographic analyses were made of luminal perfusate in two systems to confirm the identity of the biologically active component concentrated in the lumen. The first system consisted of TLC on silica gel plates[4] with the following solvent mixture: methylene chloride–methanol–benzene–formamide (20:5:5:1).

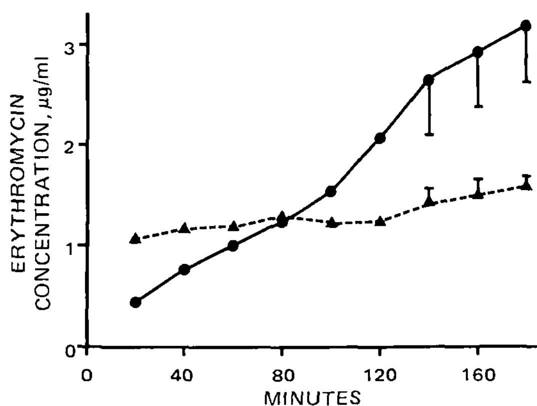

Figure 2—*Erythromycin concentration in plasma (▲) and lumen of jejunal segment (●) following vascular infusion in six animals. Standard errors of the mean are represented by vertical bars.*

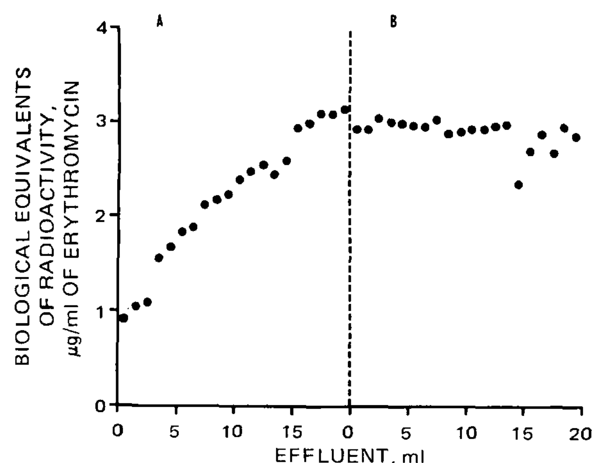

Figure 3—*Radioactivity in ultrafiltrate during saturation of filter with erythromycin (3 μg/ml) in saline (A) and following filtration of luminal buffer containing erythromycin (3 μg/ml) through saturated filter (B). See text for details of technique.*

The second system was descending paper chromatography with Whatman No. 1 paper, using a mixture of ammonium hydroxide–methyl isobutyl ketone–water (0.5:1:98.5). In the second system, three chromatographic analyses were done on samples from two animal preparations. Samples from a third animal were chromatographed in the first system.

RESULTS AND DISCUSSION

When erythromycin was infused into the jugular vein, the antibiotic concentration in the intestinal segment progressively rose and exceeded plasma concentration after 80 min (Fig. 2). The L/P ratios were significantly greater than 1 at 120 min and thereafter ($p < 0.025$), indicating a concentrative secretion of erythromycin. Preloading the luminal perfusate with five and 20 times the plasma concentration did not diminish the flux of erythromycin into the lumen (Table I). Net secretion of erythromycin into the lumen was significantly greater than zero ($p < 0.05$) at all levels of preloading.

Since the movement of erythromycin into the lumen of the intestine proceeds against a concentration gradient, passive fluxes alone are not sufficient to explain the data. At least three possible mechanisms might be responsible for this secretion: luminal trapping of erythromycin, diffusion down the transmural electrical gradient of the intestinal mucosa, and active transport.

Luminal trapping of erythromycin would involve, conceptually, diffusion into the lumen, followed by binding to some macromolecule to form a nondiffusable soluble complex. Alternatively, erythromycin in the lumen of the intestine might form an insoluble salt that could not be reabsorbed. The results of three types of experiments decrease the likelihood that luminal trapping is the mechanism of erythromycin secretion:

1. In the set of assay controls, the luminal perfusate was loaded to 1 μg/ml of erythromycin and assayed against a standard curve of erythromycin in Krebs–Ringer bicarbonate buffer solution. Zones of inhibition were of comparable size in both diluents. Any complex or insoluble precipitate would be expected to diffuse more slowly on an agar plate than would molecularly dispersed erythromycin. Therefore, the lack of assay difference between luminal perfusate and buffer solution suggests that erythromycin is freely dispersed in the luminal solution.

2. The ultrafiltration in Fig. 3 suggests that erythromycin in the luminal perfusate was not bound to large molecular weight proteins. When erythromycin in luminal buffer was filtered, 97% (2.9 μg/ml) appeared in the ultrafiltrate. Thus, erythromycin in the lu-

[2] PM30 Diaflow filter, Amacon Corp.
[3] Aquasol, New England Nuclear.
[4] Plate 5765, E. M. Laboratories, Inc.

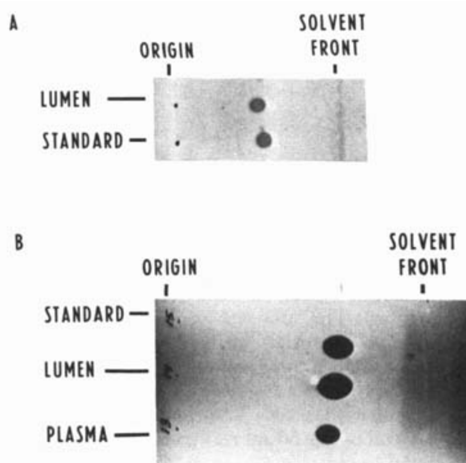

Figure 4—*Chromatography of erythromycin in luminal buffer and plasma on silica gel (A) and on Whatman No. 1 paper in a descending system (B). See text for solvent systems. Both chromatograms were developed on agar plates using* Sarcina lutea.

minal buffer is not bound in a complex with a radius larger than 29 Å.

There are two qualifications, however, to ultrafiltration data: (*a*) erythromycin was readily taken up by the ultrafiltration membrane itself, and presaturation of the membrane with erythromycin may not have fully compensated for this effect; and (*b*) binding of erythromycin to complexes smaller than 29 Å in radius would not be detected by this technique.

3. Chromatographic studies further suggested that erythromycin in luminal buffer was not complexed. In all chromatograms, only one spot of biological activity was found in the lumen as well as plasma, and this spot corresponded to erythromycin (Fig. 4). These data suggest that erythromycin in the lumen was molecularly dispersed, since an erythromycin complex would be expected to have a lower mobility than the free compound.

In view of the results of these three independent experiments, luminal trapping does not appear to be responsible for the concentrative intestinal secretion of erythromycin.

The transmural electrical potential across the intestinal mucosa must also be considered as a possible driving force for the secretion of erythromycin. In rabbit intestine *in vivo,* the transmural potential is 5.6 mv with the mucosal side negative (12).

When the flux of an ion across a semipermeable membrane is the result of simple diffusion driven by an electrical asymmetry across the membrane, the steady state of the concentration distribution can be determined from the following relationship (13):

$$\ln \frac{Ci'}{Ci''} = \frac{ZiF\,\Delta\gamma}{RT} \qquad \text{(Eq. 1)}$$

where Ci' and Ci'' are the respective concentrations on each side of the membrane, $\Delta\gamma$ is the potential difference across the membrane, Zi is the charge on the ion, F is Faraday's constant, R is the gas constant, and T is the absolute temperature.

Substituting 5.6 mv into this equation reveals that a steady-state L/P concentration ratio of only 1.23 would be expected if erythromycin were passively distributed across the intestinal mucosa due to the transmural potential alone. This predicted asymmetry is far less than the fivefold and 20-fold concentration ratios seen in tests with luminal preloading of erythromycin. Conversely, a transmural potential of 80 mv would be required to produce a 20-fold concentration asymmetry. The transmural potential, therefore, cannot be the primary energy source for erythromycin secretion.

A third possible mechanism for intestinal secretion of erythromycin is active, energy-dependent, transport into the lumen of the gut. Lee *et al.* (7, 8) found that erythromycin is actively secreted in the liver. The data in the present article strongly suggest that an active transport pathway for erythromycin is also present in the rabbit intestine. This report constitutes the first evidence for a pathway of intestinal secretion that transports erythromycin. It is not known what endogenous substances are served by this transport pathway nor what physiological function it fulfills.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 8, 1975, from the *Division of Pharmacological Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication June 12, 1975.

The authors thank Dr. W. E. Wright for his helpful suggestions in the preparation of the manuscript.

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