(5) P. P. Cohen and R. W. McGilvery, *ibid.*, **171**, 212(1947).
(6) *Ibid.*, **166**, 261(1946).
(7) P. K. Knoefel, K. C. Huang, and A. Despopoulos, *Amer. J. Physiol.*, **196**, 1224(1959).
(8) G. Bunge and O. Schmiedeberg, *Arch. Exp. Pathol. Pharmakol.*, **6**, 233(1876 77).
(9) I. Snapper, A. Gruenbaum, and J. Neuberg, *Biochem. Ztg.*, **145**, 140(1924).
(10) A. J. Quick, *J. Biol. Chem.*, **96**, 73(1932).
(11) S. H. Wan and S. Riegelman, *J. Pharm. Sci.*, **61**, 1278(1972).
(12) *Ibid.*, **61**, 1284(1972).
(13) S. H. Wan, B. von Lehmann, and S. Riegelman, *J. Pharm. Sci.*, **61**, 1288(1972).
(14) D. Schachter and J. G. Manis, *J. Clin. Invest.*, **37**, 800 (1958).
(15) D. E. Wurster and S. F. Kramer, *J. Pharm. Sci.*, **50**, 288 (1961).
(16) B. B. Brodie, J. J. Burns, and M. Weiner, *Med. Exp.*, **1**, 290 (1959).
(17) J. V. Swintosky, *J. Amer. Pharm. Ass., Sci. Ed.*, **45**, 395 (1956).
(18) G. Levy, *J. Pharm. Sci.*, **54**, 959(1965).
(19) E. L. Alpen, H. G. Mande, U. W. Rodwell, and P. K. Smith, *J. Pharmacol. Exp. Ther.*, **102**, 150(1959).
(20) E. Nelson, *ibid.*, **153**, 159(1966).
(21) H. C. Elliott, *Proc. Soc. Exp. Biol. Med.*, **121**, 861(1966).
(22) A. J. Cummings and B. K. Martin, *Nature*, **200**, 1296 (1963).
(23) A. J. Cummings, *Brit. J. Pharmacol.*, **26**, 461(1966).
(24) K. H. Froemming and W. Vollenberg, *Arbo. Pharm.*, **299**, 179(1966).
(25) "Liquid Scintillation Counting," C. G. Bell, Jr., and F. N. Hayes, Eds., Pergamon, New York, N. Y., 1958, p. 88.
(26) M. Rowland and S. Riegelman, *J. Pharm. Sci.*, **57**, 1313 (1968).
(27) P. K. Knoefel, K. C. Huang, and C. H. Jarboe, *Amer. J. Physiol.*, **1962**, 203.
(28) H. W. Smith, N. Finkelstein, L. Alimosa, B. Crawford, and M. Graeber, *J. Clin. Invest.*, **24**, 388(1945).
(29) G. Levy and J. A. Procknal, *J. Pharm. Sci.*, **57**, 1330(1968).
(30) G. Levy, L. P. Amsel, and H. C. Elliott, *ibid.*, **58**, 827(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 23, 1971, from the *School of Pharmacy, University of California Medical Center, San Francisco, CA 94122*
Accepted for publication April 12, 1973.
Supported in part by a grant-in-aid from funds of the Academic Senate Committee on Research, San Francisco Division, University of California; by a grant from the Patent Board of the University of California; and by Grants NIGMS 16496 and RR 00083 from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014
* Present address: 1 Berlin 37, Teltower Damn 31, Germany.
† Present address: Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103
▲ To whom inquiries should be directed.

Animal Models for Investigating Intestinal Drug Absorption: Various Antibiotics

DONALD PERRIER▲ and MILO GIBALDI

Abstract □ Among the most commonly used methods for studying intestinal drug absorption are the *in vitro* everted rat gut and the *in situ* rat intestinal loop. The methods were compared employing various penicillins, cephalosporins, and tetracyclines. Steady-state and, particularly, initial drug clearances across the everted rat gut and the *in situ* absorption data were not always in agreement. A comparison of these results with antibiotic absorption data in man demonstrated rank-order agreement between absorption from the *in situ* intestinal loop and human GI absorption. Based on present observations, the *in situ* intestinal loop of the rat appears suitable as an animal model for predicting human drug absorption.

Keyphrases [ ] Absorption, intestinal- *in vitro* everted rat gut and *in situ* rat intestinal loop drug permeability data, compared to antibiotic absorption data in man □ Antibiotic (penicillins, cephalosporins, and tetracyclines) intestinal absorption *in vitro* everted rat gut and *in situ* rat intestinal loop data compared to absorption data in man □ Drug permeability across intestinal membrane data from *in vitro* everted rat gut and *in situ* rat intestinal loop techniques compared to antibiotic absorption in man

A variety of *in vitro* and *in situ* animal techniques have been employed to study drug permeability across the intestinal membrane. Many of these techniques were recently discussed by Bates and Gibaldi (1). The present investigation was undertaken to compare the data obtained from two such techniques, the *in vitro* everted gut and the *in situ* intestinal loop, and to evaluate these techniques as possible models for human drug absorption. Several groups of antibiotics were studied, since this class of compounds offers a wide range in the degree to which absorption occurs in man.

METHODS

Male, Sprague–Dawley strain rats[1], weighing approximately 250 g., were fasted 14–20 hr. prior to the experiment. Water was allowed *ad libitum*.

Drug Transfer across Isolated Everted Rat Intestine—Intestinal transfer rates were determined using a modification of the method of Crane and Wilson (2). The method for preparing the everted intestine preparation was described previously (3). After severing the intestine at the pyloric junction, the first 15 cm. of intestine was discarded, the gut was everted, and the proximal portion was divided into two 10-cm. segments. The initial, proximal segment was designated Segment 1 and the distal portion was designated Segment 2.

Both segments were placed into test tubes containing approximately 100 ml. of mucosal drug solution, previously equilibrated at 37° and continually gassed with oxygen–carbon dioxide (95:5 v/v). Two milliliters of a modified physiological Krebs bicarbonate

[1] Blue Spruce Farms, Altamont, N. Y.

buffer[2] (pH 7.4) was placed inside each sac (serosal solution). In any given experiment, the initial mucosal and serosal solutions were identical except for the presence of drug in the mucosal solution; the mucosal concentration of drug remained essentially constant throughout each experiment due to its large volume. Sink conditions were maintained at all times, and the maximum serosal concentration obtained in any one experiment was less than 20% of the mucosal drug concentration. The serosal compartment was sampled every 30 min. for 2 hr. At each sampling time, the entire serosal volume was removed and then 2 ml. of buffer solution was introduced into the serosal compartment as a rinse, immediately removed, and added to the previous sample. Finally, another 2 ml. of buffer was placed into the serosal compartment for withdrawal at the next sampling interval.

A loss of functional and structural integrity of the everted gut preparation with time was reported previously (4, 5). To account for these changes, experiments were conducted where the everted intestinal preparation was incubated in drug-free buffer solution for up to 90 min. During incubation, sham samples of serosal solution were taken at 30-min. intervals and discarded. After incubation, the preparation was immediately transferred to a mucosal drug solution. The serosal compartment was sampled in the usual manner for 30 min.

In another experiment, the everted intestinal sac was incubated for 30 min. in drug-free buffer solution, at which time sham samples of serosal solution were taken and discarded. The mucosal surface was then scraped with forceps to remove the epithelial layer from the muscularis. The resulting preparation was transferred to the mucosal solution containing drug and sampled in the usual manner after 30 min.

Drug transfer rates across the everted rat intestine were expressed as mucosal-to-serosal clearances (milliliters per minute) and calculated from Eq. 1:

$$\text{clearance} = \frac{\text{amount transferred in 30 min.}}{\text{mucosal drug concentration} \times 30} \quad \text{(Eq. 1)}$$

Clearances corrected for loss of functional integrity of the everted gut preparation were calculated for sampling periods ending at 60, 90, and 120 min., denoted as Periods II, III, and IV, respectively. The clearance for the first 30 min., Period I, was used as the standard. Employment of Period I as a standard assumes that essentially no change in the functional integrity of the everted gut occurs within the first 30 min. This assumption appears valid, since Levine *et al.* (4) observed that everted gut preparations from ether-anesthetized rats show only a 10–15% loss in structural integrity in the first 60 min.

The clearance values obtained from the studies involving incubations in buffer for 30, 60, and 90 min., followed by 30 min. in drug solution, are represented by II_i, III_i, and IV_i, respectively. The increase in clearances II_i, III_i, and IV_i over Period I is attributed to a loss of functional integrity of the everted gut; when this increase is subtracted from the respective clearances for Periods II, III, and IV, the corrected clearances result. For example, to calculate the corrected clearance for Period III, III_c, Eq. 2 could be employed:

$$III_c = III - (III_i - I) \quad \text{(Eq. 2)}$$

Similarly for Periods II and IV:

$$II_c = II - (II_i - I) \quad \text{(Eq. 3)}$$

and:

$$IV_c = IV - (IV_i - I) \quad \text{(Eq. 4)}$$

When the corrected clearances for two consecutive periods were within 10% of each other, the achievement of steady-state clearance was assumed. The average of these two clearances will be referred to as the steady-state clearance.

Absorption from *In Situ* and *In Vitro* Intestinal Loops—The method of Levine *et al.* (6) was utilized to study *in situ* absorption from the rat small intestine. The animals were anesthetized with urethan, 1.3 g./kg. i.p., for the duration of the experiment. A midline incision was made, the small intestine was located, and two loops, approximately 5 cm. long, were formed. The first loop was approximately 15 cm. from the pylorus, with 1 cm. of intestine separating the two consecutive loops. One milliliter of a modified Krebs buffer solution containing drug was injected into a loop, through a ligature, by means of a syringe and blunt needle. After 30 min., the loops were excised and rinsed with normal saline. The sac was cut open at both ends, and the drug solution remaining inside the sac was permitted to run out. Approximately 25 ml. of buffer was then used to rinse out the inside of each sac.

In vitro experiments were conducted where two consecutive loops were prepared as before but, immediately prior to injection of drug solution, the loops were excised from the animal. Each loop containing drug solution was then suspended in 40 ml. of buffer solution, which was stirred at a constant rate and maintained at 37° in a jacketed beaker. At the end of 30 min., these loops were treated in the same manner as the *in situ* loops.

Before assaying the solutions obtained from the intestinal loops, each solution was passed through a 0.45-μ filter[3] to remove all solid material. Control experiments indicated that no drug was removed by this filtration step.

Drugs—The drugs examined were sodium dicloxacillin[4], sodium ampicillin[5], sodium benzylpenicillin[6], tetracycline hydrochloride[7], doxycycline hyclate[8], cephaloridine[9], and cephalexin monohydrate[10].

Assay—Cephaloridine and cephalexin were assayed polarographically using a dropping mercury electrode polarograph[11], based on the procedure reported by Benner (7). Five milliliters of 1.5 *N* sulfuric acid or 1.5 *N* sodium hydroxide was added to an equal volume of drug solution containing cephaloridine or cephalexin, respectively. The cephalexin–sodium hydroxide solution was heated at 50° for 1 hr. To both solutions, 5 drops of a nonionic detergent[12] was added as a maximum suppressor. The solutions were then deoxygenated by bubbling nitrogen through them for 5 min. Cephaloridine samples were then scanned from −0.7 to −1.2 v., while cephalexin samples were scanned from −0.5 to −1.1 v. The concentration of cephaloridine and cephalexin was related to the wave intensity, in microamperes, at −1.05 and −0.9 v., respectively.

The tetracyclines, doxycycline and tetracycline, were assayed spectrophotometrically with a spectrophotometer[13] by the method of Chatten and Krause (8). Equal volumes of 5% thorium nitrate in 0.012 *N* hydrochloric acid and tetracycline solution were combined, producing a pH 6.05 solution. The absorbance of the complex formed was measured at 395 nm.

Spectrophotometric assays were also employed for dicloxacillin, ampicillin, and benzylpenicillin. Benzylpenicillin was assayed by the method of Brandriss *et al.* (9) under the conditions outlined by Tutt and Schwartz (10). Equal volumes of a pH 2.5 glycine–hydrochloric acid buffer (0.4 *M*) containing mercuric chloride (1.6×10^{-2} *M*) and drug–buffer solution were combined, producing a final pH of 2.7. This solution was assayed at 325 nm., after standing at 25° for 100 min.

The method of Smith *et al.* (11) was employed for assaying ampicillin. A pH 4.9 citric acid (0.2 *M*)–dibasic sodium phosphate (0.4 *M*) buffer containing copper (30 mcg./ml.) was prepared. Five milliliters of pH 4.9 buffer and an equal volume of drug solution were combined, yielding a final pH of 5.2, and heated at 75° for 30 min. Absorbance was measured at 320 nm. A modified ampicillin assay was used to quantitate dicloxacillin. When equal volumes of drug solution and pH 2.2 citric acid–phosphate were mixed, a pH 2.45 solution resulted. This solution was heated at 75° for 40 min. and then assayed at 340 nm.

Microbiological assays were employed for ampicillin and dicloxacillin in the *in situ* loop experiments due to the presence of unidentified material which interfered with the chemical method. It was also decided to employ a microbiological method to check

[2] KCl, 5 m*M*; KH_2PO_4, 1 m*M*; $NaHCO_3$, 26 m*M*; and NaCl, 122 m*M*.

[3] Millipore.
[4] Bristol Laboratories, Syracuse, N. Y., Lot No. 71F1311 (D1734).
[5] Bristol Laboratories, Syracuse, N.Y., Lot No. 71F1312 (LO530).
[6] E. R. Squibb and Son, Inc., New Brunswick, N. J., Lot No. 6K877(4980).
[7] Pfizer Inc., Brooklyn, N. Y., Lot No. 0Y158–71000.
[8] Pfizer, Inc., Brooklyn, N. Y., Lot No. 11640-58002.
[9] Eli Lilly and Co., Indianapolis, Ind., Lot No. 4WM36.
[10] Eli Lilly and Co., Indianapolis, Ind., Lot No. 5BX82.
[11] Heath Built, model EUA-19-6, Heath Co., Benton Harbor, Mich.
[12] Triton X-100, 0.2%.
[13] Hitachi-Perkin-Elmer model 139, Perkin-Elmer Corp., Palo Alto, Calif.

Table I—Mucosal-to-Serosal Clearance of Various Antibiotics across the Everted Isolated Rat Jejunum

Antibiotic	Clearance, ml./min. × 10^6, ± SE^a Period I	Steady State
Benzylpenicillin (250)[b]	229 ± 36	219 ± 47
Ampicillin (500)	395 ± 54	373 ± 45
Dicloxacillin (1000)	445 ± 45	791 ± 72
Tetracycline (500)	184 ± 39	615 ± 78
Doxycycline (5000)	199 ± 32	912 ± 38
Cephaloridine (2000)	454 ± 44	749 ± 50
Cephalexin (2000)	454 ± 43	719 ± 57

[a] Mean ± standard error, five determinations. [b] Parenthetic values denote mucosal concentration in micrograms per milliliter.

Table II—Absorption of Various Antibiotics from the *In Situ* Loop of the Rat

Antibiotic	Percent Absorbed ± SE^a
Benzylpenicillin (250)[b]	15.8 ± 1.9 (6)[c]
Ampicillin (500)	22.3 ± 2.9 (5)
Dicloxacillin (1000)	30.3 ± 2.1 (6)
Tetracycline (500)	41.7 ± 0.3 (5)
Doxycycline (5000)	66.1 ± 1.6 (6)
Cephaloridine (7500)	19.4 ± 1.8 (4)
Cephalexin (7500)	53.4 ± 1.2 (3)

[a] Percent absorbed = 100 × (amount injected into loop − amount remaining in loop after 30 min.)/amount injected into loop. Mean ± standard error. [b] Parenthetic values denote initial concentration of drug in the *in situ* loop in micrograms per milliliter. [c] Parenthetic values denote number of determinations.

the *in situ* cephaloridine results obtained with the polarographic method. This procedure was followed because, following oral administration of cephaloridine to rats, virtually all of the absorbed dose was eliminated as degradation products which were believed to have been formed in the GI tract (12). It is conceivable that the polarographic method may not differentiate between cephaloridine and such degradation products. Excellent agreement was obtained between these two methods. An additional assay was not felt to be necessary for cephalexin because, following oral administration to rats, cephalexin was shown to be totally eliminated unchanged (13). For all three antibiotics, the agar well method of Bennett *et al.* (14) was used with *Bacillus subtilis* as the organism. Assays were performed in triplicate.

RESULTS

For all of the antibiotics studied, Period I clearances for the second segment of the everted gut were consistently less than the corresponding clearances for the first segment. The same general trend was observed with steady-state clearance values. This variation in clearance can probably be related to surface area differences between the two segments. However, little qualitative difference in the overall behavior of the two segments was observed. Accordingly, only data from the second segment are presented.

Mucosal-to-serosal clearances across the isolated everted gut during Period I indicate that dicloxacillin is the most permeable of the three penicillins studied, followed by ampicillin and then by benzylpenicillin (Table I). However, the differences in clearance are not very substantial. Penicillin clearances at steady state were of the same rank order as was observed for Period I, although the steady-state dicloxacillin clearance was significantly greater than Period I dicloxacillin clearance. The relative extent of penicillin absorption from the *in situ* loop (Table II) was in the same rank order as steady-state and Period I clearances.

Very little difference was seen between the Period I clearance values for doxycycline and tetracycline (Table I). However, there was approximately a 50% difference in their steady-state clearances, with doxycycline being cleared across the everted gut at a more rapid rate. The same rank order was observed in the *in situ* absorption studies (Table II) as was seen in steady-state clearance across the everted gut. In initial clearance studies with doxycycline, a steady-state clearance was not reached when a mucosal doxycycline concentration equal to the tetracycline concentration (0.5 mg./ml.) was employed. This inability to reach steady state was possibly due to continual uptake and accumulation of doxycycline in the everted gut. As a result, a doxycycline mucosal concentration significantly greater than the concentration used for tetracycline was employed to reach a steady-state doxycycline clearance.

Period I clearance values for cephaloridine and cephalexin were identical (Table I). At steady state, cephaloridine had a slightly higher clearance than did cephalexin. The everted gut clearance data for these two cephalosporins are not in agreement with *in situ* absorption results (Table II). In the latter case, cephalexin was absorbed much more extensively than cephaloridine.

In an attempt to explain the discrepancy in cephalosporin results obtained with the everted gut and *in situ* loop techniques, further studies were undertaken; the results are presented in Tables III and IV. Cephalexin traversed the intestinal membrane much more readily than did cephaloridine *in situ* (Table III). However, little difference existed between these two antibiotics in the extent to which they permeated the intestine *in vitro*, as measured by employing isolated intestinal loops. Also, there was no significant difference in the mucosal-to-serosal clearance of cephalexin and cephaloridine across the everted gut in either the presence or absence of the epithelial layer (Table IV).

DISCUSSION

Results of the present investigation demonstrated rank-order agreement between Period I and steady-state clearances across the everted gut and the *in situ* absorption data for the penicillins. Dicloxacillin was cleared at a faster rate and was absorbed more extensively than ampicillin. Of the penicillins studied, benzylpenicillin was the most slowly cleared and poorly absorbed. Period I clearance values did not differentiate either the cephalosporins or tetracyclines. However, *in situ* loop absorption results did show significant differences between doxycycline and tetracycline and between cephalexin and cephaloridine, with doxycycline and cephalexin being more extensively absorbed in their respective groups. The steady-state rank-order clearances between the two tetracyclines agreed with *in situ* absorption data. However, no difference in steady-state clearance was observed between the cephalosporins, which is contrary to the *in situ* cephalosporin results. As can be seen, *in vitro* and *in situ* methods do not agree with respect to the relative intestinal permeabilities of the various antibiotics studied.

To evaluate these two animal techniques, the *in vitro* everted gut and the *in situ* intestinal loop, with respect to their ability to serve as possible models for human drug absorption, the results obtained were compared to human absorption data from the literature. The extent to which the various antibiotics studied are absorbed from the human GI tract was calculated, employing literature urinary excretion data, using an intravenous dose as the standard.

A number of factors are involved in determining the extent to which an orally administered drug is absorbed from the GI lumen. Since the vast majority of orally administered drugs are supplied as solid dosage forms, the extent to which the drug is released from its supporting matrix becomes of prime importance. Once released, the drug's solubility and stability in solution must be considered. Resistance to degradation and lack of interaction with components present in the GI tract are desirable. The drug must also possess suitable physical properties that enable it to permeate the GI membrane. One or more of these factors appears responsible for rendering the majority of the antibiotics studied in the present investigation incompletely absorbed from the GI lumen.

In most literature reports surveyed, human GI absorption was studied employing solid dosage forms. This raises the problem of potential dissolution rate-limited absorption rather than permeability rate-limited absorption. However, in our view, the antibiotics studied would be expected to be permeability rather than dissolution rate limited in their absorption since they are freely soluble in water.

Benzylpenicillin is very susceptible to acid hydrolysis and attack by penicillinase in the GI tract (15, 16). Both processes compete for drug at the absorption site. Ampicillin, which is relatively stable in acid and neutral solutions (15, 17), would on a stability basis be expected to be and, in fact, is more extensively absorbed than benzylpenicillin (Table V) even though it does not appear to be

Table III—Transfer of Cephalosporins out of Rat Jejunum Loops

Cephalosporin	Percent Transferred ± SE[a]	
	In Situ	*In Vitro*
Cephaloridine (7500)[b]	19.4 ± 1.8 (4)[c]	18.2 ± 2.3 (4)
Cephalexin (7500)	53.4 ± 1.2 (3)	24.1 ± 1.6 (3)

[a] Percent transferred = 100 × (amount injected into loop − amount remaining in loop after 30 min.)/amount injected into loop. Mean ± standard error. [b] Parenthetic values denote initial concentration of drug in loop in micrograms per milliliter. [c] Parenthetic values denote number of determinations.

Table IV—Effect of Epithelial Layer Removal on Clearance of Cephalosporins across the Everted Isolated Rat Jejunum

Cephalosporin	Clearance, ml./min. × 10^6, ± SE[a]	
	Control	Epithelial Layer Removal
Cephaloridine (2000)[b]	712 ± 23	832 ± 24
Cephalexin (2000)	748 ± 22	761 ± 47

[a] Mean ± standard error, five determinations. [b] Parenthetic values denote mucosal concentration in micrograms per milliliter.

resistant to penicillinase (18). However, it should not be implied that if a particular penicillin derivative is not subject to GI degradation it will be 100% absorbed. For example, dicloxacillin appears resistant to both acid-catalyzed hydrolysis and degradation by penicillinase (19), but considerably less than 100% of this drug appears to be absorbed (Table V).

The rank order obtained with respect to the steady-state penicillin clearance across the everted gut and absorption from the *in situ* intestinal loops is in agreement with the relative extent of penicillin absorption in humans (Tables I, II, and V). Benzylpenicillin had the lowest clearance and percent absorbed of the three penicillins studied, followed by ampicillin and then dicloxacillin. The everted gut studies (notably steady-state studies) as well as *in situ* studies suggest that the penicillins do indeed have different membrane permeability characteristics. At normal intestinal pH as well as the pH of the everted gut studies, the carboxyl group of the penicillins would be essentially totally dissociated, which may partly explain the generally poor permeability of the penicillins. With ampicillin, the negative charge associated with the carboxyl group would be partially balanced by the positive charge associated with the amino group, which has a pKa of 7.25 (20), resulting in an ampicillin molecule with a net partial negative charge rather than a total negative charge. This difference in overall polarity of these two penicillins may aid in explaining why ampicillin is more permeable than benzylpenicillin. Dicloxacillin, even though totally dissociated, appears to have a substantially more lipophilic substituent than the α-aminophenyl and phenyl substituents of ampicillin and benzylpenicillin, respectively. Such an increase in lipophilicity may account for the increased permeability of dicloxacillin over the other two penicillins.

The cephalosporins are structurally very similar to the penicillins but are much more stable than the penicillins (17). Cephalexin and ampicillin are identical in structure except for the basic differences in the cephalosporin and penicillin nuclei, but cephalexin is essentially totally absorbed on oral administration (21). Higher steady-state clearance values and increased absorption from the *in situ* intestinal loops were observed for cephalexin as compared to ampicillin (Tables I and II). These permeability differences can be attributed to the variation in the basic nuclei of the penicillin and cephalosporin molecules.

Although cephalexin is extensively absorbed, cephaloridine is very poorly absorbed (1–2%) in human subjects (22). This lack of absorption has been attributed to the presence of the pyridinium group (23). *In vitro* clearance values suggest that there is no significant difference in the permeability characteristics of cephalexin and cephaloridine (Table I). This is totally inconsistent with *in situ*[14] rat and human absorption results (Tables II and V), and an attempt was made to explain this anomaly. Initially, the extent to which the cephalosporins were transported across *in vitro* intestinal loops was studied. Cephalexin and cephaloridine permeated the *in vitro* loop to comparable degrees, even though substantial differences were observed *in situ* (Table III).

In vivo and *in situ* the epithelial cell of the intestinal villus constitutes the only anatomical barrier of significance controlling the movement of drug from the lumen of the gut into the body. Once past this barrier, the transferred drug gains ready access to either blood or lymph capillaries. Therefore, *in vivo* and *in situ* the process of intestinal absorption is governed by properties of the epithelial barrier and the rates of blood and lymph flow. However, *in vitro* a drug must traverse the epithelium plus the underlying connective and muscle tissue. As a result, the rate-limiting barrier for *in vitro* transport might be the intestinal musculature, a barrier that would not be encountered in *in vivo* and *in situ* transfer. The *in situ* and *in vitro* intestinal loop results in the present investigation suggest that the muscle and connective tissue serve as significant barriers to cephalexin transfer resulting in both cephalosporins being cleared or absorbed to similar extents *in vitro*. Further support for this postulate are the results of a study where clearance measurements were made across the everted gut from which the epithelial layer had been removed, leaving only the underlying muscle layer and connective tissue. There is relatively little change in the mucosal-to-serosal clearance after removal of the epithelial layer, and no significant difference was observed in the clearance values either before or after the everted gut was stripped of the epithelial layer (Table IV). These results suggest that the epithelial layer is probably not the primary barrier to cephalosporin transport *in vitro* and that the muscle layer serves as an equal barrier to both cephalexin and cephaloridine *in vitro*. Based on these results, the everted gut technique appears unsuitable as a model for human drug absorption in the case of the cephalosporins.

The initial clearance values (Period I) of doxycycline and tetracycline are essentially identical. However, steady-state clearances suggest that doxycycline is considerably more permeable than tetracycline (Table I). Furthermore, this increase in permeability of doxycycline over tetracycline is in agreement with absorption data from the *in situ* loop studies (Table II). The difference in permeability of these two tetracyclines is consistent with observed differences in their chloroform–water partition coefficients. Doxycycline was shown to have a significantly higher partition coefficient than tetracycline over the 2–8 pH range (24). It is generally accepted that doxycycline is better absorbed than tetracycline in man (25), and studies in the literature support such claims. An analysis of oral and intravenous plasma level data, presented recently by Leibowitz *et al.* (26), suggests that 85% of an oral dose of doxycycline is absorbed. This study supports the data of Fabre *et al.* (27), where 93% of an oral doxycycline dose was reported to be absorbed. These findings can be compared to 77% for tetracycline (Table V). Differences in absorption are further supported by studies where doxycycline and tetracycline have been administered with food. Food appears to have little influence on doxycycline absorption (28–30) while significantly depressing the oral absorption of tetracycline (31). Furthermore, dissolution studies[15] conducted in this laboratory indicate that doxycycline hyclate has approximately a 3.5 times greater dissolution rate than tetracycline hydrochloride. This finding may also contribute to better absorption of doxycycline.

CONCLUSIONS

Based on the results of the present investigation, *in vitro* methods, where the drug must traverse both the epithelial and underlying connective tissue and muscle layers rather than just the epithelial layer to permeate the intestinal membrane, should be employed as models for GI absorption only with extreme caution. Period I clearances across the everted gut yield very limited insight into human GI absorption. Although the rank order in Period I clear-

[14] Due to limitations in assay sensitivity, initial cephalosporin concentrations in the *in situ* loop were substantially greater than the initial mucosal concentrations employed in the everted gut studies, 7.5 *versus* 2 mg./ml. Based on one run at a mucosal concentration of 7.5 mg./ml., the clearances of both cephalosporins across the everted gut were the same as the clearances obtained when a mucosal concentration of 2 mg./ml. was employed.

[15] Rotating-disk method as described by Levy and Sahli (32) using a precision speed control apparatus (33), modified Krebs bicarbonate buffer, 37°, and a rotation speed of 60 r.p.m.

Table V—Absorption of Various Antibiotics following Oral Administration to Humans

Antibiotic	Percent Absorbed[a]	Reference[b]
Benzylpenicillin	18	31, 34
Ampicillin	35	35, 36
Dicloxacillin	64	34, 37
Tetracycline	77	38
Doxycycline	93	27, 30
Cephaloridine	2	22
Cephalexin	83	21, 39

[a] Amount eliminated in urine after oral administration as a percent of amount eliminated in urine after an equal intravenous dose. [b] Literature reference from which human data was obtained.

ances of the penicillins agrees with human absorption data, relatively small differences in clearances were observed as compared to human studies. Moreover, Period I clearance values did not differentiate either the cephalosporins or the tetracyclines. Steady-state clearance measurements did yield a reasonable rank-order estimate of the relative extent to which the penicillins and tetracyclines were absorbed in man. However, steady-state clearances seriously failed with the cephalosporins. Even where appropriate, steady-state clearance studies require much more time and are more difficult to run than are *in situ* loop studies.

The *in situ* loop technique proved to be a very suitable animal model for predicting antibiotic absorption in man. Within each group of antibiotics, the same rank order was obtained between the extent of absorption from the *in situ* loop and the human GI lumen. The *in situ* intestinal loop technique also serves as a reasonable model for absorption even when all of the antibiotics studied are considered as a single group. With the exception of cephaloridine, excellent rank-order agreement was obtained between *in situ* loop and human studies. The *in situ* loop studies indicate more extensive absorption of cephaloridine than would be expected. This may be due to an inherent error in the technique since the amount of drug leaving the loop lumen is measured and not the amount of drug that actually reaches the blood supply. Therefore, extensive accumulation of cephaloridine in the intestinal tissue may account for the apparent overestimate in the extent of cephaloridine absorption from the intestinal loop.

REFERENCES

(1) T. R. Bates and M. Gibaldi, in "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, p. 57.

(2) R. K. Crane and T. H. Wilson, *J. Appl. Physiol.*, **12,** 145 (1958).

(3) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196,** 296(1970).

(4) R. R. Levine, W. F. McNary, P. J. Kornguth, and R. LeBlanc, *Eur. J. Pharmacol.*, **9,** 211(1970).

(5) M. Gibaldi and B. Grundhofer, *Proc. Soc. Exp. Biol. Med.*, **141,** 564(1972).

(6) R. M. Levine, M. R. Blair, and B. B. Clark, *J. Pharmacol.*, **114,** 78(1955).

(7) E. J. Benner, *Antimicrob. Ag. Chemother.*, **1971,** 201.

(8) L. G. Chatten and S. I. Krause, *J. Pharm. Sci.*, **60,** 107(1971).

(9) M. W. Brandriss, E. L. Denny, M. A. Huber, and H. G. Steinman, *Antimicrob. Ag. Chemother.*, **1963,** 626.

(10) D. E. Tutt and M. A. Schwartz, *J. Amer. Chem. Soc.*, **93,** 767(1971).

(11) J. W. G. Smith, G. E. deGrey, and V. J. Patel, *Analyst*, **92,** 247(1967).

(12) H. R. Sullivan and R. E. McMahon, *Biochem. J.*, **102,** 976 (1967).

(13) H. R. Sullivan, R. E. Billings, and R. E. McMahon, *J. Antibiot.*, **22,** 195(1969).

(14) J. V. Bennett, J. H. Brodie, E. J. Benner, and W. M. M. Kirby, *Appl. Microbiol.*, **14,** 170(1966).

(15) F. P. Doyle, J. H. C. Nayler, H. Smith, and E. R. Stove, *Nature*, **191,** 1091(1961).

(16) L. Weinstein, in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1970, p. 1204.

(17) M. A. Schwartz and F. H. Buckwalter, *J. Pharm. Sci.*, **51,** 1119(1962).

(18) P. G. Gooding, C. A. Fernandez, J. Perche de Menezes, and J. Ximenes, *Curr. Ther. Res.*, **14,** 43(1972).

(19) V. P. Naumann and B. Kempf, *Arzneim.-Forsch.*, **15,** 139 (1965).

(20) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **58,** 1510(1969).

(21) R. L. Zabransky, M. A. Gardner, and J. E. Geraci, *Mayo Clin. Proc.*, **44,** 876(1969).

(22) J. W. Kislak, B. W. Steinhauer, and M. Finland, *Amer. J. Med. Sci.*, **251,** 433(1966).

(23) C. H. O'Callaghan and S. M. Kirby, *Postgrad. Med. J., Suppl.*, **46,** 9(1970).

(24) M. Schach von Wittenau and R. Yeary, *J. Pharmacol. Exp. Ther.*, **100,** 258(1963).

(25) H. J. Simon, S. J. Yaffe, V. J. Fontana, and S. G. Axline, *Antimicrob. Ag. Chemother.*, **1967,** 121.

(26) B. J. Leibowitz, J. L. Hakes, M. M. Cahn, and E. J. Levy, *Curr. Ther. Res.*, **14,** 820(1972).

(27) J. Fabre, E. Milek, P. Kalfopoulos, and G. Merier, *Schweiz. Med. Wochenschr.*, **101,** 593(1971).

(28) P. Lee, E. R. Crutch, and R. B. I. Morrison, *N. Z. Med. J.*, **75,** 355(1972).

(29) J. R. Migliardi and M. Schach von Wittenau, *Proc. Int. Congr. Chemother., 5th*, **2,** 165(1967).

(30) J. E. Rosenblatt, J. E. Barrett, J. L. Brodie, and W. M. M. Kirby, *Antimicrob. Ag. Chemother.*, **1967,** 134.

(31) J. G. Wagner, L. G. Leslie, and R. S. Gove, *Int. J. Clin. Pharmacol.*, **2,** 44(1969).

(32) G. Levy and B. A. Sahli, *J. Pharm. Sci.*, **51,** 58(1962).

(33) G. Levy and W. Tanski, Jr., *ibid.*, **53,** 679(1964).

(34) L. W. Dittert, W. O. Griffen, Jr., J. C. LaPiana, F. J. Shainfeld, and J. T. Doluisio, *Antimicrob. Ag. Chemother.*, **1970,** 42.

(35) J. O. Klein and M. Finland, *Amer. J. Med. Sci.*, **245,** 544 (1963).

(36) T. C. Eickhoff, J. W. Kislak, and M. Finland, *ibid.*, **249,** 163(1965).

(37) E. A. DeFelice, *J. Clin. Pharmacol.*, **7,** 275(1967).

(38) C. M. Kunin, *Proc. Soc. Exp. Biol. Med.*, **110,** 311(1962).

(39) J. A. Davies and J. M. Holt, *J. Clin. Pathol.*, **25,** 518(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 2, 1973, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York, Buffalo, NY 14214*

Accepted for publication May 24, 1973.

Supported in part by a grant from the Consumer Products Group, Warner-Lambert Research Institute; by GRS Grant FR-5-501RR-05454010, National Institutes of Health; and by a studentship for D. Perrier from the Medical Research Council of Canada.

▲ To whom inquiries should be directed.