Transfer of Clindamycin and 1′-Demethyl-4′-depropyl-4′-pentylclindamycin by the Cannulated-Everted Rat Gut

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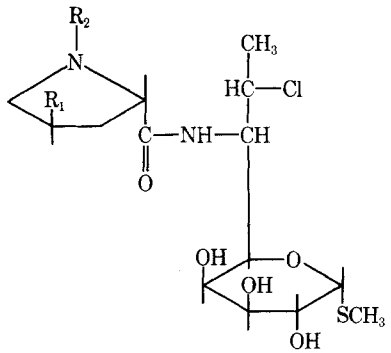
Abstract □ A cannulated-everted rat gut technique was used to measure the rate of clindamycin transfer to the serosal side for various mucosal concentrations of clindamycin. The rate of transfer of 1′-demethyl-4′-depropyl-4′-pentylclindamycin to the serosal side for a given 1′-demethyl-4′-depropyl-4′-pentylclindamycin mucosal concentration can be calculated from the clindamycin data if the difference in pKa between clindamycin and 1′-demethyl-4′-depropyl-4′-pentylclindamycin is taken into account. The predicted rate of transfer for 1′-demethyl-4′-depropyl-4′-pentylclindamycin is approximately one-third of the transfer rate for clindamycin and is in good agreement with the observed rate of transfer. It was assumed that only the unionized species of both compounds were transferred by similar mechanisms. No correction was made for lipid solubility, since the true partition coefficients (ether/water) for the two compounds are similar. GLC was used to assay for both compounds as their trifluoroacetate derivatives.

Keyphrases □ Clindamycin, transfer—in cannulated-everted rat gut □ 1′-Demethyl-4′-depropyl-4′-pentylclindamycin, transfer—in cannulated-everted rat gut □ Transfer rates—clindamycin, 1′-demethyl-4′-depropyl-4′-pentylclindamycin, in cannulated-everted rat gut □ GLC—analysis

It is generally believed that for most compounds only the unionized species is absorbed from the gastrointestinal tract (1–3). It has also been shown that partitioning of drugs between gastric juice and plasma is dependent on their pKa's (3). To determine whether the pH-partition hypothesis could be used predictively in the assessment of rates of transfer of ionizing compounds through biological membranes, two compounds dissimilar in pKa but similar with respect to other physical properties are needed. It is well known that compounds with higher relative lipid solubilities are generally better absorbed. Since the effect of relative lipid solubility on transfer rate has not been quantitated, compounds with similar true partition coefficients[1] are needed to make the assumption that lipid solubility would not have an appreciable effect on the transfer rate. In addition, compounds of similar molecular weight and structure are needed to assure that these compounds would be transferred by similar mechanisms with diffusion constants of similar magnitude.

Two compounds that meet these requirements fairly well are clindamycin[2] (Compound 1) and 1′-demethyl-4′-depropyl-4′-pentylclindamycin (Compound 2). Their free base molecular weights are 427 and 441, respectively. These two compounds also have similar true partition coefficients (ether/water) but different pKa's. As will be shown in this paper, correcting the concentrations of Compound 1 and Compound 2 for the amount unionized, which equals amount unprotonated, allows the prediction of the transfer rate for Compound 2 from that of Compound 1 when the cannulated-everted rat gut technique is used.



	R_1	R_2
clindamycin	C_3H_7	CH_3
1′-demethyl-4′-depropyl-4′-pentylclindamycin	C_5H_{11}	H

EXPERIMENTAL

General—Clindamycin (Compound 1) and 1′-demethyl-4′-depropyl-4′-pentylclindamycin (Compound 2) were prepared in the Upjohn Research Laboratories. Trifluoroacetic anhydride was from Eastman Kodak, white label. Tetrahydrofuran was purified and stored over alumina. All other reagents were analytical grade.

Cannulated-Everted Rat Gut Procedure—Krebs-Ringer phosphate–bicarbonate buffer was prepared according to the literature (4), except the $CaCl_2$ concentration was decreased 50% and the buffer was made 10 m*M* in glucose. The pH of the buffer at 37° in equilibrium with 95% O_2–5% CO_2 was 7.20. After 2 mg. base equivalent of the hydrochloride salt of clindamycin was added per milliliter of buffer, the pH was 7.18.

Sprague-Dawley rats, weighing 180–200 g., were killed by a blow on the head. A segment of intestine starting at the bile duct entrance was stripped from the mesentery, rinsed, and maintained as far as possible in cold, oxygenated, Krebs-Ringer phosphate–bicarbonate buffer. A 10-cm. segment was measured by hanging a 2-g. weight on the distal end from the bile duct. The segment was everted on a polyethylene cannula, with the distal end tied to a groove in the cannula (5). One milliliter of buffer was syringed into the sac, and the sac was placed into 75 ml. of oxygenated buffer at 37° containing the drug to be transferred. This large volume on the mucosal side of the intestine acted as a reservoir of drug, so the effective concentration of drug to be transferred did not change significantly with time. The entire preparation of the intestine was usually completed within 6 min. of the animal's death. Peristaltic action was present throughout the experiment.

After 30 min., serosal fluid was withdrawn by inserting a small diameter polyethylene tube, attached to a syringe, down the lumen of the cannula and aspirating. The serosal side was washed twice with 1.5 ml. of buffer at 37°; the washings, along with the sample, were collected in a centrifuge tube. One milliliter of buffer was then added to the serosal side to obtain a second 30-min. transfer sample.

After the second 30 min. of transfer, the sac was removed from the drug solution and the mucosal side (outside) was rinsed with 6 ml. of buffer. The sac was placed over a centrifuge tube, and a hole was cut

[1] The true partition coefficient equals the concentration of unionized species in the organic phase/the concentration of the unionized species in the aqueous phase.

[2] Cleocin, The Upjohn Co.

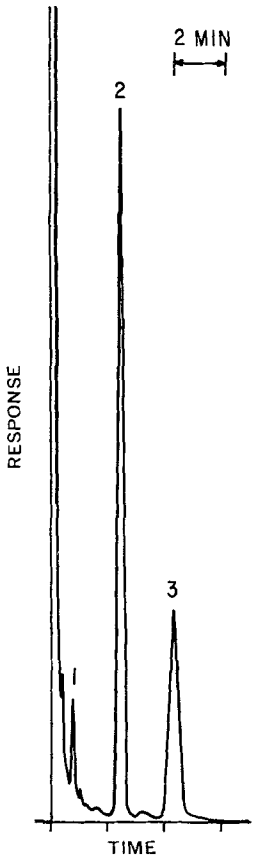


Figure 1—*Typical gas chromatogram for clindamycin. Peak 1 = impurities extracted from buffer and rat intestine. Peak 2 = internal standard: triphenylmethane. Peak 3 = clindamycin trifluoroacetate derivative.*

in the bottom to allow the serosal solution to drain. The serosal side was rinsed with 4 ml. of buffer and collected in the centrifuge tube for assay.

The experimental range of error between rats for the aspirating-sampling technique was ±23% when it was used for the second 30-min. sample, while the error for the cut-rinse-sampling technique was only ±10%. Due to this difference in experimental error, the cut-rinse-sampling technique was used to collect the data for the comparison of transfer of Compounds 1 and 2.

GLC Assay—Trifluoroacetate derivatives of Compounds 1 and 2 were used for GLC because they are readily formed, they are stable in an excess of reagent, they can be chromatographed at lower temperatures than silyl ethers or acetates, and there is no residue build-up on the detector.

For Compound 1, 2 ml. of chloroform containing 0.2 mg. or 0.02 mg. of triphenylmethane as internal standard was added to the buffer containing 0.1–1 mg. or 0.01–0.1 mg. of clindamycin base, respectively. To aid extraction, 0.2 ml. of 0.1 *N* KOH was added. The mixture was shaken vigorously and centrifuged. Most of the chloroform layer was transferred to a 2-ml. volumetric flask and evaporated to dryness under a dry stream of air. The dried residue was dissolved in 0.2 ml. of tetrahydrofuran, and 0.2 ml. of trifluoroacetic anhydride was added. The mixture was allowed to react for 30 min. at room temperature and then was gas chromatographed on a U-shaped glass column 0.61 m. × 1.27 cm. (2 ft. × 0.5 in.) packed with Gas Chrom Q, 60–80 mesh, and coated with 1% OV-17. Oven temperature was 178° and helium gas flow was 40 ml./min. A typical gas chromatogram is given in Fig. 1 and standard curve in Fig. 2. The experimental range due to the assay is ±5%.

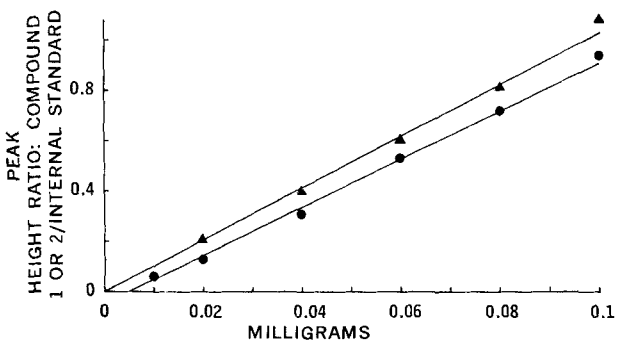


Figure 2—*Standard curve for: ▲, Compound 1; and ●, Compound 2.*

For Compound 2, 4 ml. of chloroform containing 0.06 mg. of tetraphenylethylene as internal standard was added to the buffer containing 0.01–0.1 mg. of Compound 2 as clindamycin base equivalents. After the mixture was shaken vigorously and centrifuged, it was assayed in the same manner as Compound 1, except the mixture was allowed to react for 30 min. at 47°. A typical gas chromatogram is given in Fig. 3 and standard curve for the major component of the *cis–trans*-isomer in Fig. 2. The extracted impurities from buffer and rat intestine cause no interferences in either assay.

RESULTS AND DISCUSSION

Determination of pKa and Partition Coefficient—The Radiometer titrator was used to determine the pKa's of 7.45 ± 0.02 and 7.96 ± 0.04 ($\bar{X} \pm \sigma$) for Compounds 1 and 2, respectively, at 37° in aqueous media.

The true partition coefficient for Compound 1 between diethylether and water is 9.8 at 25° (6). The apparent partition coefficient of Compound 2 between diethylether and water was measured at pH 7.70, 25°, and the true partition coefficient was calculated (7) to be 7.2. These partition coefficients for Compound 1, 9.8, and Compound 2, 7.2, are sufficiently similar and their pKa's sufficiently dissimilar to test the transfer hypothesis.

Cannulated-Everted Rat Gut Transfer of Compound 1—All weights for Compounds 1 and 2 are in terms of clindamycin base equivalents.

The transfer rate of Compound 1 was measured for glucose concentrations of 0, 10, and 20 m*M*. The transfer rate of Compound 1 in the absence of glucose was approximately twice the rate when glucose was present. With no glucose, peristaltic action was not observed and large sections of villi started to slough off during the first 30-min. interval, indicating this increase in transfer rate may be due

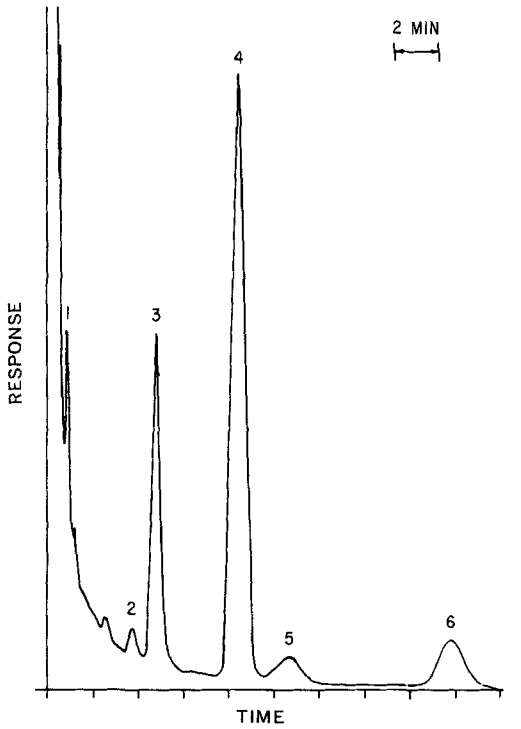


Figure 3—*A typical gas chromatogram for Compound 2. Peaks 1, 5, and 6 = impurities extracted from buffer and rat intestine. Peak 2 = minor component of* cis–trans *mixture of Compound 2. Peak 3 = major component of* cis–trans *mixture of Compound 2. Peak 4 = internal standard: tetraphenylethylene.*

Table I—Milligrams of Clindamycin Transferred for Three Consecutive 30-min. Intervals[a]

Rat	First 30 min.	Second 30 min.	Third 30 min.
	Milligrams		
1	0.035	0.167	0.330
2	0.054	0.192	0.238
3	0.058	0.245	0.257
4	0.040	0.271	0.244

[a] Samples obtained by aspirating technique.

to intestine degeneration. Since the intestine rapidly degenerates without glucose and 10 or 20 m*M* glucose gave equivalent transfer rates, the experiments were run using 10 m*M* glucose.

The amount of clindamycin base transferred in the second 30-min. interval was the same, within experimental error (range ±10%), for the first seven successive 10-cm. segments of intestine proximal to the bile duct. Thus, the first 10-cm. segment proximal to the bile duct from different rats was used in the rest of the experiments.

When the mucosal bathing solution contained 1 mg. clindamycin base/ml. of buffer, 0.041 ± 0.01 and 0.189 ± 0.02 ($\bar{X} \pm \sigma$) mg. of clindamycin base were transferred to the serosal side during the first (sampled by aspirating) and second (sampled by cutting and rinsing), 30-min. intervals, respectively. This difference in amount transferred is related to the lag time required to establish a steady state. To ensure that a steady state had been established by the second 30-min. interval, the aspirating-sampling technique was used to measure three 30-min. samples on the same intestinal segment. The results for four different rats are given in Table I. In all cases but one, the third 30-min. sample was within the experimental range (±23%) of the second 30-min. sample. This indicates that a steady state had been established for the second 30-min. sample. Since by the end of 1.5 hr., small particles of tissue were starting to appear in the bathing solution, the transfer rates for the second 30-min. interval were used in all calculations.

The amount transferred per second 30-min. interval curve for various mucosal concentrations of Compound 1 is linear within experimental error, as shown in Fig. 4. Each point is an average of at least four values, and the bars indicate the experimental range ±10% by the cut-rinse-sampling technique.

Predicted and Measured Transfer Rates for Compound 2—Due to its poor aqueous solubility, only 0.875 mg. of Compound 2 as clindamycin base per milliliter of buffer was used for the mucosal solution. At this experimental concentration, 0.875 mg./ml., only 0.057 ± 0.007 ($\bar{X} \pm \sigma$) mg. of Compound 2 as clindamycin base was transferred to the serosal side during the second 30-min. interval. Data indicate steady state had been reached.

To calculate the amount of Compound 2 that is expected to be transferred using the Compound 1 curve, the following equation is used:

$$\text{pKa} = \text{pH} - \log \frac{A}{HA_T - A} \qquad \text{(Eq. 1)}$$

where A = amount of unionized compound, and ($HA_T - A$) = amount of ionized compound or the total amount of compound added, HA_T, minus A.

The equations:

$$HA_T = 2.79A_{\text{Compound 1}} \qquad \text{(Eq. 2)}$$

$$HA_T = 6.75A_{\text{Compound 2}} \qquad \text{(Eq. 3)}$$

for Compounds 1 and 2, respectively, are obtained by substituting the pKa's and pH of the buffer at 37° into Eq. 1. When the total concentration bathing the mucosal side is the same for both compounds, Eqs. 2 and 3 are combined to give:

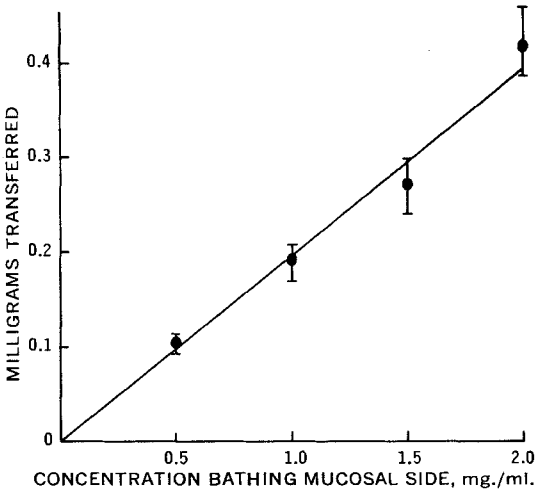


Figure 4—*Amount of clindamycin base transferred during the second 30-min. interval* versus *its mucosal concentration.*

$$A_{\text{Compound 2}} = 0.413A_{\text{Compound 1}} \qquad \text{(Eq. 4)}$$

or the effective concentration of Compound 2 is approximately four-tenths that of Compound 1. The effective Compound 2 concentration for 0.875 mg./ml. at pH 7.2 was calculated with Eq. 4 as 0.36 mg./ml. Using this effective concentration of 0.36 mg./ml. with Fig. 4, it was predicted that 0.070 ± 0.007 mg. of Compound 2 as clindamycin base should be transferred in the second 30-min. interval when 0.875 mg./ml. of Compound 2 was in the mucosal solution. The experimentally observed value of 0.057 ± 0.007 is in good agreement with this predicted value of 0.070 ± 0.007.

The experimentally observed value differs from the predicted value in the direction that is expected if the relative lipid solubilities are considered, and it appears that the *in vitro* partitioning between ether and water simulates the biophase partitioning. But due to the experimental error (±12%) involved, further corrections of the predicted value are unwarranted. If no correction was made for pKa, the predicted value for the amount to be transferred was 0.172 mg. in the second 30-min. interval, which is much higher than the observed values. Thus, it appears that transfer rates can be predicted for compounds differing in pKa if the unionized concentration is quantitated and certain other criteria, such as similar molecular weight, structure, and lipid solubility, are met.

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