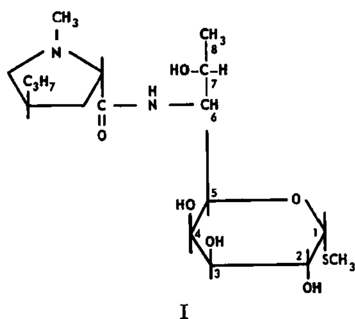
Absorption of Lincomycin and Lincomycin Esters from Rat Jejunum

By H. PATRICK FLETCHER, HEATHER M. MURRAY, and TODD E. WEDDON

The absorption of lincomycin equivalents from rat intestinal loops was improved when short-chain (3 and 4 carbons) fatty acid esters of the drug were used. The 7-butyrate and 7-propionate esters of lincomycin appear to be absorbed more efficiently than corresponding 2-esters. *In vivo* and *in vitro* experiments indicate that this difference in absorption results from intestinal hydrolysis of the 2-esters. The first-order rate constants for the disappearance of the esters from the intestinal loops were obtained from an equation which expresses the disappearance of lincomycin as the sum of two first-order expressions.

THE INCOMPLETE ABSORPTION of lincomycin (I) from the rat gastrointestinal tract was



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indicated by results of Meyer and Lewis (1). It was estimated that 45% of the lincomycin disappeared from the small intestine after oral dosing and that most of the absorption occurred in the first quarter of the small intestine.

Fatty acid esters of lincomycin were synthesized in an attempt to find a tasteless derivative for a pediatric preparation. The monoesters used in this study were formed at the 2- and 7-positions and were hydrochloride salts. Since the addition of the fatty acid moiety increased the lipid solubility of the lincomycin molecule (2), the absorption from the small intestine was investigated. Results reported in the literature (3, 4) have indicated that increased lipid solubility may play an important role in the absorption of certain drugs.

The object of this study was to find an ester or analog which would be more efficiently absorbed from the intestine and result in higher serum antibiotic levels. Thus, the absorption of these compounds from ligated loops was studied in the rat jejunum.

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EXPERIMENTAL

Animals—Male, albino rats of the Sprague-Dawley strain weighing between 180–210 g. were used throughout this study. Food and water were available to the animals up to the time of surgery. Five rats were used for each time interval in the intestinal absorption studies.

Drug Solutions—The drug solutions were prepared so that 0.5 ml. yielded 6.50 μmoles of lincomycin base. The compounds were placed into distilled water, and no suspending agents or solubilizers were used.

Intestinal Loops—The method for preparing intestinal loops in the rat jejunum was described by Levine and co-workers (5). Under ether anesthesia, a midline incision was made, and the first 10 cm. of the jejunum were exposed. After the lumen of the exposed jejunum had been cleared, a proximal ligature was loosely placed around the intestine and a distal ligature secured 10 cm. below it. Care was taken so that the ligatures did not interfere with the major blood vessels supplying the intestinal loop. The drug solutions were placed into the lumen of the loop *via* a 1-ml. syringe attached to a 26-gauge needle after the proximal ligature had been tightened around the needle. After delivery of the drug solution, the needle was withdrawn and the proximal ligature was completed. The intestine was replaced into the abdomen, the incision closed, and the rat was allowed to recover.

At the end of appropriate time intervals, the rats were again given ether anesthesia, sacrificed by decapitation, and blood samples were taken. The intestinal loops were removed and individually homogenized in saline with glass homogenizers. The homogenized loops from rats given lincomycin HCl were placed into volumetric flasks and brought to a 100-ml. volume with 0.1 *M* phosphate buffer (pH 7.9).

The antibiotic activity of the lincomycin esters are much less than lincomycin HCl. Since the amount of lincomycin ester remaining in the loop was determined by the microbiological assay for lincomycin base, it was necessary to hydrolyze the esters by subjecting such loops (as the homogenates) to alkaline hydrolysis. The homogenized loops from rats given the lincomycin derivatives were quantitatively placed into 20-ml. beakers and subjected to alkaline hydrolysis with 0.4 *N* NaOH over steam

for 5 min. After the hydrolysis, the homogenates were cooled, adjusted to pH 7.9–8.0 with HCl, and brought to a volume of 100 ml. with 0.1 *M* tris buffer (pH 7.9). Preliminary experiments demonstrated that the hydrolysis procedure did not degrade lincomycin as determined by the microbiological assay.

The amount of lincomycin or lincomycin derivatives originally placed into the loops was also determined by microbiological assay. The drug solution, 0.5 ml., was placed into 3 ml. of homogenates of jejunum from nontreated rats and subjected to the same procedures as given above for loops from experimental rats. Five of these spiked homogenates were prepared for each absorption study.

In Vitro Hydrolysis by Intestinal Homogenates—Segments of jejunum were taken from nontreated rats immediately after sacrifice and homogenized with chilled Kreb's Ringers bicarbonate solution containing glucose (5 g./l.). Three milliliters of homogenate, equivalent to 0.5 g. of intestine, was placed into 25-ml. conical flasks. Control flasks received the 3 ml. of homogenate which had been coagulated by heat. After 0.5 ml. of drug solution containing 2.65 mg. of lincomycin base equivalents of the lincomycin ester was deposited into each flask, it was placed into the Dubnoff metabolic shaker for appropriate time intervals. The flasks were incubated under a constant pressure of 95% O_2 and 5% CO_2 at 37°. At the end of the incubation period, all homogenates were coagulated with heat and the contents were brought to appropriate volume using a 0.1 *M* phosphate buffer pH 7.9. The amount of hydrolysis was estimated by an increase in microbiological activity from the intrinsic activity level observed in the control flasks.

Statistical Treatment of Data—The intestinal absorption of the esters were compared using semilog plots based on the disappearance of lincomycin from the intestinal loop. A regression analysis was used to determine the slopes of the absorption plots. These slopes were compared to lincomycin HCl and the significance of the differences was tested by analysis of covariance. The computations for these studies were obtained by use of the IBM 1620 computer. The curve resulting from nonlinear regression analysis was completed by the IBM 360.

Microbiological Assay—The microbiological assay for lincomycin in the serum described by Vavra *et al.* (6) was used for both serum and homogenates. The test organism was *Sarcina lutea* grown in Penassay seed agar. The samples from the absorption study of a particular ester were assayed on the same day. The results were corrected for day to day assay variation using the spiked homogenates and the theoretical content of lincomycin base.

RESULTS AND DISCUSSION

Absorption from Intestinal Loops—The absorption of lincomycin HCl from the loops was repeated four times during the study. The absorption plots were reproducible and the slopes were not significantly different when compared by analysis of covariance. A representative lincomycin absorption plot is shown in Fig. 1.

The semilog plots (Fig. 1) indicated that the absorption of the 2-monoesters of lincomycin increased as the chain length of the fatty acid group decreased. Also, unlike the absorption of lincomycin HCl, this figure shows that the absorption of the esters was rapid at the early time intervals and much slower in the later intervals. Thus, the semilog plots of these means resulted in a curved line, especially for the 2-butyrate and 2-propionate esters. A possible explanation for this curvature will be given later.

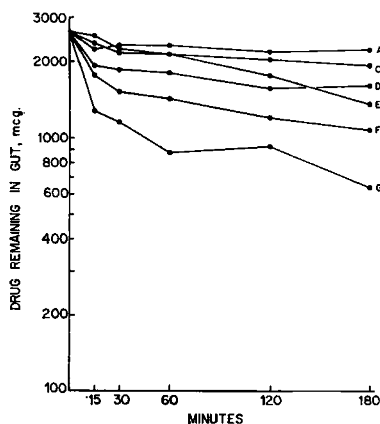

Fig. 1—Absorption of lincomycin and lincomycin esters from jejunal loops of rats. Each point is a mean of five rats. Key: A, 2-laurate; C, 2-octanoate; D, 2-hexanoate; E, linco HCl; F, 2-butyrate; G, 2-propionate.

By taking the results (mcg. remaining in loop *versus* time) in the early time intervals prior to the "leveling off" of the absorption curve, the slopes of the esters were compared to lincomycin HCl in the same time intervals.

The increased slopes (Table I) from the regression analysis in the early time intervals appear to correspond with the percent dose absorbed after 2 hr. The analysis of covariance indicated that 2-butyrate, 7-butyrate, 2-propionate, and 7-propionate esters are significantly better absorbed (as reflected by the slopes) than lincomycin HCl (Table I).

The results in Table I also show the increase in lipid solubility resulting from the addition of the fatty acid groups to the lincomycin molecule. It appears that as far as the esters are concerned, the amount absorbed decreases as the lipid solubility increases. This may be a result of the decreased aqueous solubility of the longer chain esters. This decreased aqueous solubility may partially explain the poor absorption of the 2-laurate and 2-octanoate, since a precipitate was noted when they were placed in loops or intestinal homogenates. Thus, the better absorbed esters probably possess a balance between an increased lipid solubility and an adequate aqueous solubility.

The 7-butyrate and 7-propionate esters were better absorbed than their corresponding 2-esters (Table I). The comparison of the 2-butyrate and 7-butyrate esters (Fig. 2) revealed that the means in the later time intervals of the 2-butyrate absorption run parallel to lincomycin HCl. This was also apparent when the slopes in the later time intervals were not significantly different when compared by analysis of covariance. This parallelism in the

TABLE I—ABSORPTION OF LINCOMYCIN ESTERS

Lincomycin Compd.	pH[a]	Initial Slope[b]	Results of Analysis of Covariance[c]	Intrinsic Partition Coefficients[d]	Apparent % Absorbed at End of 2 hr.
Lincomycin HCl	5.7	−0.0015	—	0.06	33
2-Laurate HCl	3.8	−0.0009	0.05	7,800	17
2-Octanoate HCl	3.9	−0.0017	N.S.	2,750	27
2-Hexanoate HCl	5.0	−0.0051	N.S.	390	40
2-Butyrate HCl	4.6	−0.0084	0.05	40	54
2-Propionate HCl	4.4	−0.0120	0.01	14	64
7-Butyrate HCl	5.2	−0.0094	0.01	4	82
7-Propionate HCl	5.2	−0.0170	0.01	1	91

[a] pH of drug solutions placed in loops. [b] Based on mcg. remaining in the intestine at 0, 15, and 30 min. [c] Statistically compared to lincomycin HCl. [d] Ether–water: values were reported by W. Morozowich and F. Young (personal communication).

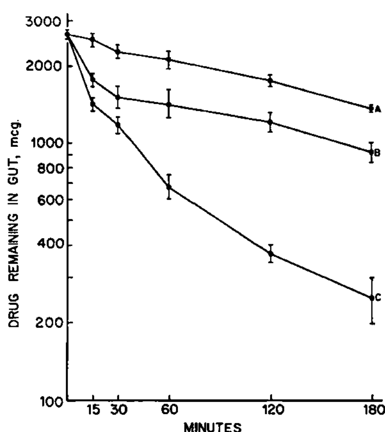

Fig. 2—Absorption of 2- and 7-butyrate from rat jejunal loops. (Each point represents a mean of five rats ± SE.) Key: A, linco HCl; B, linco-2-butyrate; C, linco-7-butyrate.

later part of the absorption plots was also found for the 2-propionate ester. These results indicate that lincomycin HCl was being absorbed in the later time intervals after the ester had been placed into the loop. This leads to the investigation of the hydrolysis of the ester by the intestinal enzymes.

In Vitro Hydrolysis—The increase in antibiotic activity from the control flask level (Table II) was assumed to be due to the presence of lincomycin base resulting from hydrolysis of the less active ester. Even though hydrolysis rates as such were not obtained due to the presence of antibiotic activity in the control flasks, it is obvious from Table II that the 7-esters, butyrate and propionate, are more resistant to the hydrolysis of intestinal enzymes than their corresponding 2-esters. The differing hydrolysis of the 2- and 7-butyrates in homogenates was also confirmed by gas chromatographic results. By measuring the resulting lincomycin peaks, approximately four times as much lincomycin was noted in the case of the 2-butyrate which confirmed the results obtained with microbiological assay. Also, the apparent resistance of the 7-butyrate to hydrolysis was demonstrated *in vivo* when intestinal loops were removed 30 min. after dosing and a microbiological assay was run before and after alkaline hydrolysis. The pre- and posthydrolysis antibiotic activities of the lincomycin-2-butyrate loops were not significantly different. However, the posthydrolysis antibiotic activity for the 7-butyrate ester was four times that of the prehydrolysis activity and this difference was statistically significant. Although the absorption of the 7-hexanoate was not studied, its hydrolysis in intestinal homogenates revealed again the apparent protection from hydrolysis afforded by the 7-position.

Serum Levels—The serum levels (Table III) reflected the amount of absorption from the intestinal loops as far as the 2-esters are concerned. The results also indicate that the serum levels for the better absorbed 2-esters appear to reach higher levels earlier than lincomycin HCl. These higher levels were especially apparent when lincomycin-2-butyrate and lincomycin-2-propionate were placed into ligated loops.

The antibiotic serum levels obtained after the more efficiently absorbed 7-esters were no higher than lincomycin HCl. An explanation for these results may be that a portion of the lincomycin in the serum was present as the 7-ester which is essentially inactive in the assay. Alkaline hydrolysis of serum from animals with lincomycin-7-butyrate and 7-propionate in ligated loops increased the antibiotic activity (Table IV). It is probable that this increase in antibiotic activity resulted from the hydrolysis of 7-esters to the active lincomycin.

Lincomycin-7-propionate was identified in the serum of rats which had the ester in intestinal loops. This identification was made by mass spectrometric results from chloroform extracts of the rat serum.

TABLE II—HYDROLYSIS OF LINCOMYCIN ESTERS BY HOMOGENATES OF RAT INTESTINE

Derivative	mcg.[a] Placed into Flask	Intrinsic Activity, mcg. in Control Flask[b]	Lincomycin, mcg. after Incubation
2-Butyrate	2,280 ± 28[c]	421 ± 2	2,220 ± 48
7-Butryate	2,050 ± 68	60 ± 6	610 ± 62
2-Propionate	2,640 ± 10	400 ± 18	2,630 ± 45
7-Propionate	2,700 ± 56	<15	<15

[a] Determined by microbiological assay. [b] Contained boiled homogenate. [c] Mean of six flasks ± *SE.*

Table III—Serum Levels of Lincomycin Activity (mcg./ml.) at Various Time Intervals

Lincomycin Derivatives	Postdose, min. 15	30	60	120	180
Lincomycin HCl	0.4[a]	1.3	0.6	0.5	0.3
2-Laurate	0.8	0.5	<0.3	<0.3	<0.3
2-Octanoate	0.7	0.7	0.4	<0.3	<0.3
2-Hexanoate	1.6	0.9	0.6	0.5	<0.3
2-Butyrate	2.1	1.2	0.8	0.6	<0.3
2-Propionate	4.7	1.9	1.0	0.3	<0.3
7-Butyrate	1.3	1.0	0.4	<0.3	<0.3
7-Propionate	1.0	0.9	0.5	<0.3	<0.3

[a] Mean of 5 rats.

The hydrolysis of serum from rats receiving lincomycin HCl and lincomycin-2-butyrate in ligated loops did not increase the antibiotic activity in the serum. These results indicated that the increased antibiotic activity was not a result of hydrolysis of some inactive conjugate of lincomycin.

If the 7-ester were present in the serum, then the ester, as such, was absorbed from the intestine and the enzymes outside the intestine must also have a decreased ability to hydrolyze the 7-esters. Studies with liver homogenates indicated that the enzymes in the liver also have a decreased ability to hydrolyze the 7-butyrate ester when compared with the 2-butyrate ester.

Stephens and Conine (7) reported increased blood levels resulting from oral administration of fatty acid esters of erythromycin to humans. As in the lincomycin study, the highest levels were obtained with the propionate and butyrate esters.

Effect of pH of Drug Solution—The pH of the various solutions of esters used in this study varied from 3.8 to 5.2 (Table I). No effect on absorption was detected when the 2-propionate solution was adjusted from 4.4 to 6.0, when the 2-octanoate was adjusted from 3.8 to 4.9, and when lincomycin HCl was adjusted from 5.0 to 3.0.

Proposed Model for Intestinal Absorption—The proposed model for the absorption of the lincomycin esters assumes that the esters are better absorbed than lincomycin HCl. This increased absorption probably results from increased lipid solubility as reflected by the intrinsic partition coefficients of the ester, or perhaps other properties which were not examined in this study. Thus, the amount of lincomycin or its ester absorbed depends upon how well the ester resists hydrolysis and remains in the ester form while in the intestinal loop (Fig. 3). Evidence presented thus far *in vivo* and *in vitro* indicates that this could explain the difference in absorption between the 2- and the 7-esters.

Table IV—Lincomycin Serum Levels in Hydrolyzed and Nonhydrolyzed Serum

Postdose, min.	mcg./ml.[a] 15	30	60	120	180
7-Butyrate					
Nonhydrolyzed	1.3	1.0	0.4	<0.3	<0.3
Hydrolyzed	2.2	1.8	1.0	0.3	<0.3
7-Propionate					
Nonhydrolyzed	1.0	0.9	0.5	<0.3	<0.3
Hydrolyzed	2.8	1.8	1.2	0.3	<0.3

[a] Results from the pooling of samples from five rats.

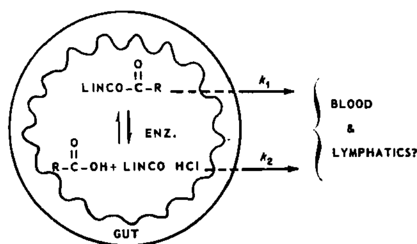

Fig. 3—Diagram for the proposed model for the absorption of lincomycin esters.

The equation for the proposed model is

$$y = x_1 e^{-k_1 t} + x_2 e^{-k_2 t}$$

where

k_1 = first-order rate constant for the disappearance of lincomycin ester
k_2 = first-order rate constant for the disappearance of lincomycin HCl
x_1 = amount of lincomycin ester
x_2 = amount of lincomycin HCl
t = time
y = amount of lincomycin in intestine

This equation states that the amount of drug remaining in the intestine (y) is the sum of two first-order expressions representing the disappearance of two different compounds from the intestine. The first-order rate constants, k_1 and k_2, were estimated from the absorption curves (Fig. 1) for the particular 2-esters. The k_2 constant was estimated from a line drawn through the means in the later time intervals. The k_1 was estimated from plotting the residuals in the first three time intervals of the absorption curve. It was assumed that this constant represented the estimate of the constant for the disappearance of the lincomycin ester from the intestine. Thus, k_2 was used as the rate constant for the disappearance of lincomycin HCl.

These rough estimates for k_1 and k_2 and the individual rat data were used in the computer program which calculated the k_1 and k_2 based on nonlinear regression analysis. This is a modification of a method described by Wagner and Metzler (8).

The 2-propionate curve calculated by the above method by IBM computer is shown in Fig. 4. The

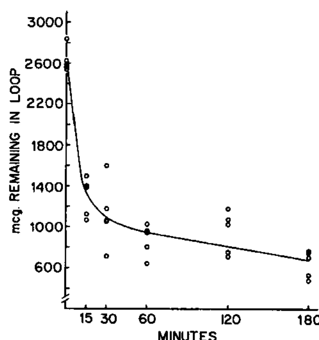

Fig. 4—Calculated curve (using nonlinear regression analysis) for the absorption of lincomycin-2-propionate. Key: O, observed data; ——, computer-calculated curve.

TABLE V—ESTIMATES OF FIRST-ORDER RATE CONSTANTS[a] FOR THE DISAPPEARANCE OF LINCOMYCIN AND LINCOMYCIN ESTER

	k_1[b], 95% C.L.	k_2,[c] 95% C.L.
Lincomycin-2-propionate	8.32 (2.29–14.35)	0.17 (0.05–0.30)
Lincomycin-2-butyrate	6.06 (0.00–12.4)	0.13 (0.01–0.25)
Lincomycin-2-hexanoate	9.67 (0.00–20.9)	0.06 (0.02–0.11)

[a] Obtained from nonlinear regression analysis. [b] k_1 rate constant for ester. [c] k_2 rate constant for lincomycin.

first-order rate constants for the better absorbed 2-ester are shown in Table V. Even these calculated rate constants must be considered estimates, since this equation does not take into consideration the rate of enzymatic hydrolysis in the intestine. The amounts of lincomycin base and the ester in the intestine are not only affected by the disappearance of each through absorption, but also by the amount of ester being hydrolyzed. However, even with these limitations, this method allows one to estimate k_1 and k_2 when the semilog absorption plots are not linear. Because of the linearity of its semilog absorption plots (Fig. 1), the rate constant for the absorption of lincomycin HCl was calculated by the usual method using the slope obtained by linear regression analysis. This rate constant was 0.21 hr.$^{-1}$ for lincomycin HCl. The estimates for lincomycin HCl are lower in Table V, 0.17 hr.$^{-1}$, 0.13 hr.$^{-1}$, and 0.06 hr.$^{-1}$.

This model and the data presented in this study indicate that the 2-ester and its hydrolysis product, lincomycin HCl, are both absorbed at the early time intervals, while only lincomycin HCl is absorbed in the later time intervals, the ester being completely hydrolyzed.

SUMMARY

The absorption of lincomycin was improved by using derivatives of lincomycin which consisted of esters of short-chain fatty acids (3 and 4 carbons). The absorption efficiencies of the various esters were modified by intestinal hydrolysis. The explanation for absorption of these esters is based on a model which states that the lincomycin ester and lincomycin base both contribute to this absorption.

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Keyphrases

Lincomycin HCl, esters—absorption
Jejunum, rat—ligated loops
Absorption, lincomycin HCl, esters—intestinal loops
Model, intestinal absorption—lincomycin HCl, esters
Intestinal homogenates—lincomycin HCl, esters hydrolysis

Ultraviolet Spectrum Correlations with the Conjugate Acid-Base Species of Acetarsone and Arsthinol

By C. F. HISKEY and F. F. CANTWELL

The ionization constants of acetarsone (pK_1 = 3.73, pK_2 = 7.9, pK_3 = 9.3) and arsthinol (9.5 ± 0.1) have been determined potentiometrically in aqueous solution and the UV spectra of the individual conjugate species have been deduced. Assignment of the second ionization step of acetarsone to the phenolic proton is made. Hydrolytic atmosphere oxidative cleavage of arsthinol in alkaline aqueous solution to produce acetarsone has been demonstrated and spectrophotometric assay methods for these two substances are proposed.

ACETARSONE (3-acetamido-4-hydroxy phenylarsonic acid) and arsthinol (cyclic 3-hydroxypropylene ester of 3-acetamido-4-hydroxy dithiobenzene arsenous acid) have the following structures:

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