Metabolism of Clindamycin II:
Urinary Excretion Products of Clindamycin in Rat and Dog

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Abstract ☐ The urinary excretion products of clindamycin metabolism in rats and dogs were isolated and characterized. Of the radioactive materials excreted in rat urine, 53% was identified as unchanged clindamycin, 31% as clindamycin sulfoxide, and 15% as N-demethylclindamycin. In dog urine, 36% of the radioactivity excreted was identified as unchanged drug, 28% as the sulfoxide, 28% as clindamycin glucuronide, and 9.16% as N-demethylclindamycin. No glucuronide conjugate was found in the rat urine. Thus, based on the metabolic patterns found in the urine of the two species, sulfoxidation and N-demethylation were the predominant metabolic routes in the rat, while conjugation with glucuronic acid and sulfoxidation were the major pathways in the dog.

Keyphrases ☐ Clindamycin metabolism—urinary excretion products in rats and dogs ☐ Excretion, urinary—identification of clindamycin metabolites, rats, dogs

In the first paper of this series (1), studies on the absorption and excretion of a new antibiotic, clindamycin[1], in experimental animals were described. In continuation of the study of clindamycin metabolism, effort was directed to the elucidation of the metabolic pathways for clindamycin in mammals. This report describes the isolation and characterization of urinary excretion products in the rat and the dog.

Studies of Brodasky *et al.* (2) on the characterization of human urinary excretion products of clindamycin suggested that N-demethylclindamycin was the major metabolite. These authors also observed two other drug-related substances with chromatographic behavior similar to clindamycin sulfoxide and clinimycose (the 1-hydroxy derivative). The trace amounts of these two components precluded their isolation and characterization. Clindamycin sulfoxide and N-demethylclindamycin (3) have been isolated and identified as the metabolic products of clindamycin in microorganisms.

EXPERIMENTAL[2]

Dosage and Collection of Samples—Twelve female Sprague-Dawley rats (Upjohn strain) were given 100-mg./kg. oral doses of clindamycin hydrochloride through a stomach tube every day for a month. Tritium-labeled drug (6.0 μc.) was given on the first and the last days to check for possible adaptation or stimulation of metabolism and to provide labeled metabolites for isolation. Urine specimens were collected once a day through stainless steel metabolism cages and frozen immediately until used. The rats were maintained on powdered food[3] and deionized water. Food was

given during the daytime only to provide empty stomachs before dosing every morning. Water was provided at all times.

Three male beagle dogs, weighing 8.2, 8.9, and 11.7 kg., respectively, each received single daily oral doses of 500 mg. clindamycin hydrochloride (in hard-filled capsules) for 7 days. Urine samples were collected once a day through stainless steel metabolism cages. Food[4] was given once a day and deionized water was allowed freely. Urine samples containing radioactive clindamycin and metabolites from the previous experiment (1) were added to the pooled urine to monitor the separation.

Preparation of Extract—The polystyrene resin was thoroughly washed successively with acetone, methanol, and distilled water before being packed into a 38 × 300-mm. glass column. A pooled urine sample, spiked with radioactive urine (total volume 2 l.), was filtered through glass wool and adjusted to pH 8.5. The filtered urine was passed through the column. The column was subsequently washed with 6 l. of distilled water and eluted with: (a) 2 l. of a methyl ethyl ketone-water mixture (95:5), and (b) 2 l. of methanol. The spent urine and aqueous wash were discarded. The ketone and methanol eluents were pooled and evaporated to dryness. Recovery of radioactivity was greater than 90%.

The drug-related components in the urine extracts were examined by TLC. The separation procedure is presented in Scheme 1.

Isolation of Clindamycin and Metabolites from Rat Urine Extract—*Countercurrent Distribution*—The dried material from rat urine was dissolved in 20 ml. of anhydrous methanol and acidified with methanolic hydrochloric acid. Twenty milliliters of acetone and 400 ml. of anhydrous ether were added, and the mixture was cooled to 0°. A large amount of precipitate, containing essentially all of the radioactivity, separated out and was collected by filtration. The precipitate was dissolved in 30 ml. of water-saturated n-butanol and loaded into the first three tubes of a 500-tube, all-glass countercurrent distribution separator with 10 ml. of n-butanol as the upper phase and 10 ml. of water as the lower phase. After 500 transfers were completed, aliquots were withdrawn from every 10th tube and analyzed for radioactivity. The locations of radioactivity (Fig. 1) were then determined, and the rest of the tubes were emptied and combined into two fractions. The two layers in each fraction were separated. The pH of the aqueous layer was adjusted to 10 with 1 N NaOH before being extracted three times with equal volumes of n-butanol. The extracts were combined with each corresponding fraction, and the pooled butanol solution was evaporated to dryness. Extracted aqueous layers were checked for radioactivity losses before being discarded. The losses of radioactivity in aqueous layers were negligible. Drug-related components in each fraction were examined by TLC.
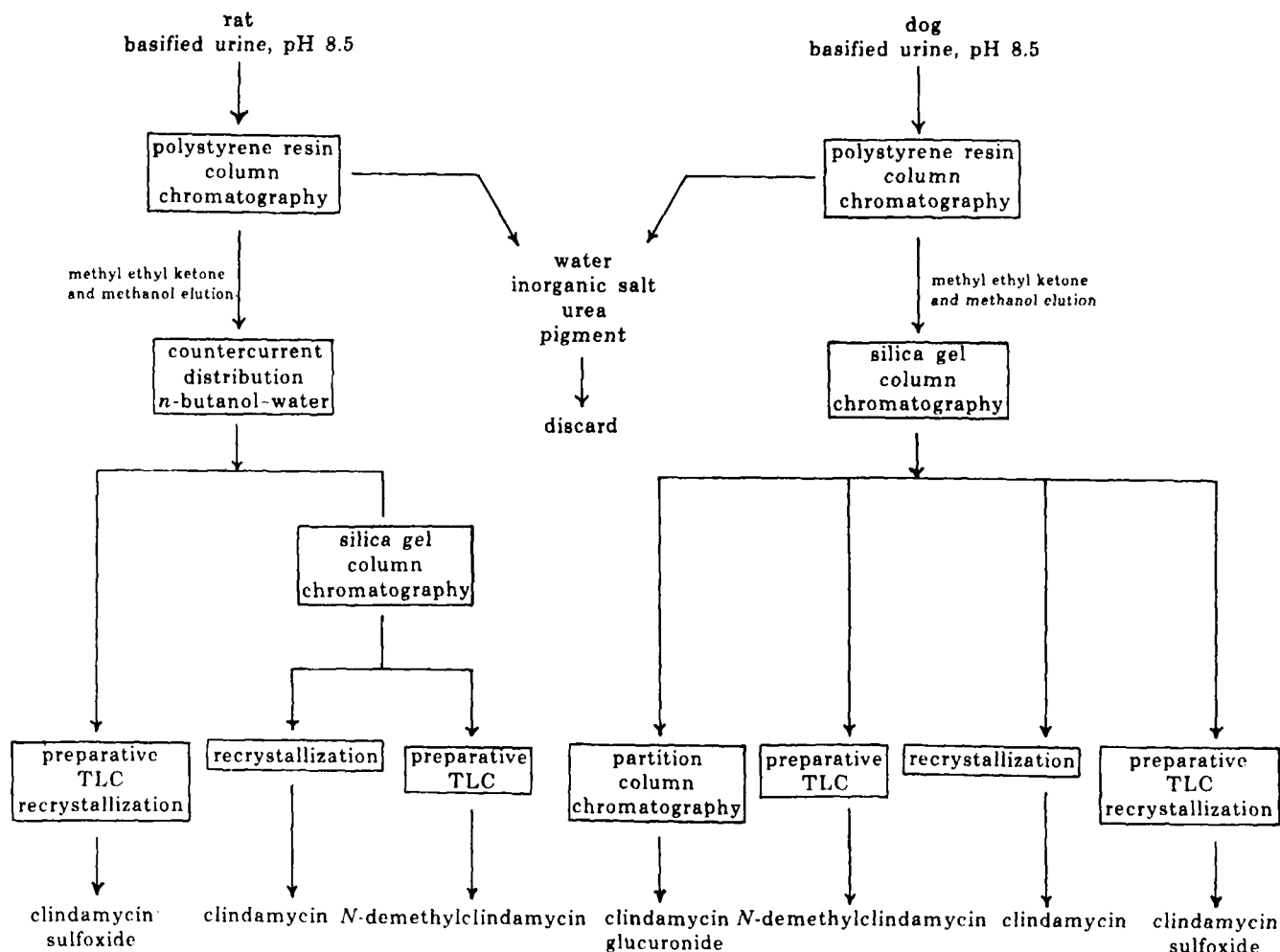
Purification of Clindamycin Sulfoxide—The fraction containing clindamycin sulfoxide (Fraction I-CCD) was dissolved in 1 ml. of methanol, and the entire fraction was streaked on a 2-mm. thick preparative thin-layer silica gel G_{254} plate. The plate was developed in the methyl ethyl ketone–acetone–water (93:26:10) solvent. Standard clindamycin sulfoxide was spotted and run along with the streak to identify the desired band. After the solvent front reached the top, the band corresponding to the clindamycin sulfoxide was scraped off and homogenized in 100 ml. of absolute methanol. The silica gel was then filtered out and washed twice with 50-ml. aliquots of methanol. The combined methanol solution was evaporated to dryness, and the residue was leached twice with 5-ml. aliquots of chloroform–methanol (4:1). Traces of silica gel at this point could be removed by centrifugation. The chloroform–methanol solution was concentrated and the entire preparative TLC procedure was repeated once with the same solvent and once with chloroform–methanol (6:1) as the developing solvent. The final product, showing

rat
basified urine, pH 8.5

dog
basified urine, pH 8.5

polystyrene resin
column
chromatography

polystyrene resin
column
chromatography

water
inorganic salt
urea
pigment

discard

methyl ethyl ketone
and methanol elution

methyl ethyl ketone
and methanol elution

countercurrent
distribution
n-butanol-water

silica gel
column
chromatography

silica gel
column
chromatography

preparative
TLC
recrystallization

recrystallization

preparative
TLC

partition
column
chromatography

preparative
TLC

recrystallization

preparative
TLC
recrystallization

clindamycin
sulfoxide

clindamycin

N-demethylclindamycin

clindamycin
glucuronide

N-demethylclindamycin

clindamycin

clindamycin
sulfoxide

Scheme 1—Sequential experimental steps for the separation of clindamycin and metabolites from rat and dog urine

only one spot on the thin-layer plate, was crystallized from ethanol and ethyl acetate.

Purification of Clindamycin and N-Demethylclindamycin—The fractions from countercurrent distribution (tubes in Fraction II-CCD) containing these two components were pooled, evaporated to dryness, and redissolved (or suspended) in 20 ml. of chloroform–methanol (10:1). This solution was placed on the top of a silica gel (0.02–0.5 mm.) column packed in the same solvent (26 × 240 mm.). Elution was carried out first with 250 ml. of chloroform–methanol (10:1), then with 400 ml. of chloroform–methanol (5:1), and finally with 250 ml. of chloroform–methanol (2:1). Eluates were collected in 10-ml. fractions, and aliquots were withdrawn from every other tube for radioactivity counting. The desired fractions were combined, concentrated, and analyzed by TLC.

clindamycin hydrochloride
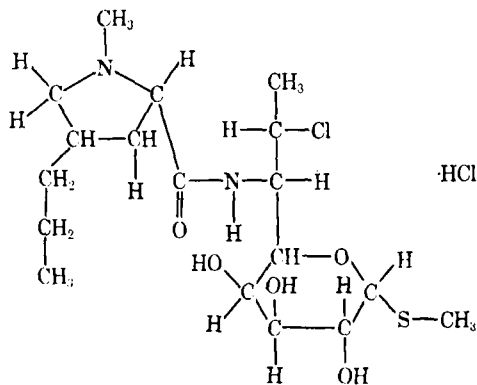
Fractions containing clindamycin were evaporated to dryness and redissolved in 1 ml. of methanol. The drug was converted to the hydrochloride salt and precipitated by adding 1 ml. of acetone and 20 ml. of ether. The precipitate was crystallized from 2 ml. of ethanol and 7 ml. of ethyl acetate. The final product appeared to be free from contaminants and was analyzed by NMR, IR, mass spectrometry, and TLC.

Fractions containing N-demethylclindamycin were concentrated and purified by preparative TLC according to the procedure used for clindamycin sulfoxide. The final product was evaporated to dryness and analyzed without crystallization.

Isolation of Clindamycin and Metabolites from Dog Urine Extract —In the dog study, since there was one radioactive zone located near the origin on the thin-layer chromatogram (Fig. 2), it was separated first by silica gel column chromatography. The dried material, obtained from polystyrene resin chromatography, was homogenized in a solvent mixture of chloroform–methanol (95:5) and loaded on a silica gel column (38 × 360 mm.) packed in the same solvent. Elution was carried out successively with the following solvent mixtures: (a) 700 ml. chloroform–methanol (20:1), (b) 500 ml. chloroform–methanol (10:1), (c) 500 ml. chloroform–methanol (8:1), (d) 500 ml. chloroform–methanol (5:1), (e) 1000 ml. chloroform–methanol (1:3), (f) 600 ml. methanol, and (g) 500 ml. methanol–water (1:1). The eluates were collected in 10-ml. fractions. An aliquot from every third fraction was analyzed for radioactivity. Eight radioactivity fractions were separated (Table I) (Fig. 3), concentrated, and analyzed by TLC.

Purification of Unchanged Clindamycin and Clindamycin Sulfoxide —The purification of intact clindamycin (Fraction I) and clindamycin sulfoxide (Fraction II) was carried out by preparative TLC according to the methods described in the previous section. Clindamycin was recrystallized as the hydrochloride salt from a mixture
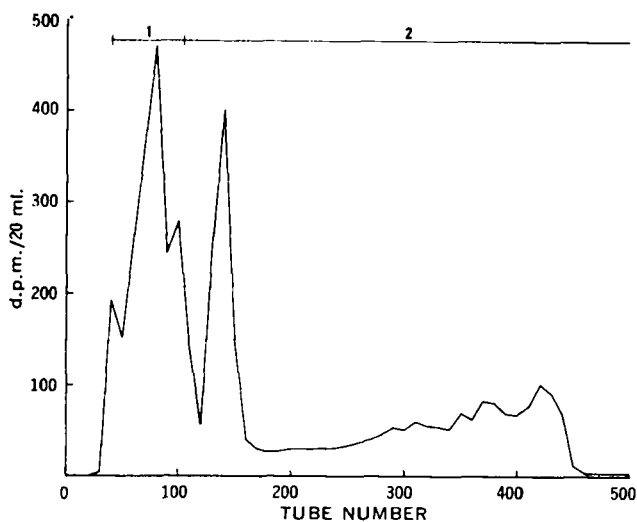
Figure 1—*Countercurrent distribution of rat urine extract.*

of ethyl acetate–ethanol (7:2). Clindamycin sulfoxide was isolated as the free base. Both compounds were identified by NMR, IR, and mass spectrometry.

Purification of Clindamycin Glucuronide—When eluted from the silica gel column, fractions 4 and 5 (Fig. 3) were contaminated with large amounts of a yellow urinary pigment. These fractions were further purified by liquid–liquid partition chromatography. A solvent mixture of 300 ml. of n-butanol, 700 ml. of ethyl acetate, and 400 ml. of water was mixed by shaking and allowed to settle. The upper phase and lower phase were separated. Twenty grams of an amorphous diatomaceous silica filter aid[b] were mixed with 200 ml. of the upper phase. The lower phase (8 ml.) was added dropwise with vigorous stirring. The mixture was poured into a column and allowed to settle. The fraction to be purified was: (a) dissolved in 2 ml. of the lower phase, (b) mixed with 4 g. of Hyflo Super Cel, and (c) diluted with 10 ml. of the upper phase; the eluate was collected in 10-ml. fractions. Aliquots from every other tube were counted for radioactivity. The radioactive zone was pooled and evaporated to dryness. The same procedure was repeated twice to obtain about 500 mg.
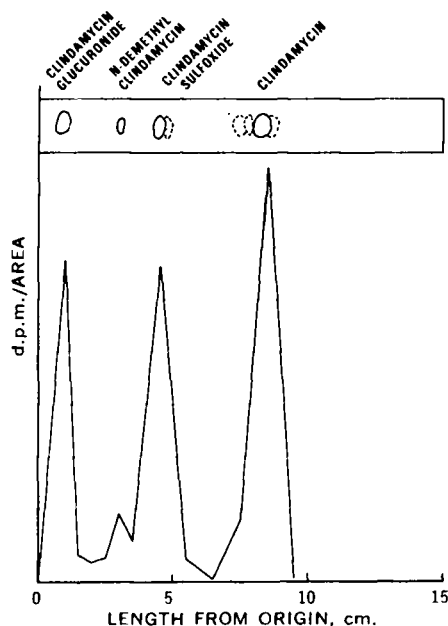

Figure 2—*Quantitative thin-layer chromatogram of pooled dog urine extract (unhydrolyzed). The solvent system was ethyl acetate–acetone –water (8:5:1).*

[b] Dicalate.

Table I—Recovery of Radioactivity after Silica Gel Chromatography of Dog Urine Extract[a]

Fraction	Microcuries	Recovery, %	Identification
1	3.28	23.39	Clindamycin
2	5.64	40.22	Clindamycin sulfoxide
3	0.95	6.73	N-Demethylclindamycin
4	0.486	3.46	Clindamycin glucuronide
5	3.161	22.54	Clindamycin glucuronide
6	0.142	1.01	—
7	0.153	1.09	—
8	0.068	0.49	—

[a] Total microcuries added = 14.022 μc. Total percentage recovery = 98.93%.

of white fluffy material. TLC analyses showed that a single zone was present. The material was used for spectroscopic analyses.

Additional clindamycin glucuronide was purified by countercurrent distribution, using a solvent system of n-butanol–water. Based upon TLC, the clindamycin glucuronides purified by countercurrent distribution and partition chromatography were identical.

Analytical Methods[6]—*TLC*—TLC, used for monitoring the separation procedure, was carried out on 2.5 × 20-cm. glass plates precoated with 0.25 mm. of silica gel G_{254}. The following solvent systems were used: (a) methyl ethyl ketone–acetone–water (93:26: 10), (b) chloroform methanol–water (8:4:0.4), (c) chloroform–methanol (6:1), (d) n-butanol–water (5:1), (e) ethyl acetate–acetone–water (8:5:1), and (f) acetone chloroform (3:1). Since clindamycin glucuronide remained near the origin when chromatographed in these solvent mixtures, three additional systems were required. They were: (g) methyl ethyl ketone acetone–water (1:3:1), (h) ethyl acetate–acetone–water (2:6:1), and (i) chloroform–methanol (1:3).

Spraying reagents used to locate the desired spots were as follows:

Spraying Reagent	Reaction
8.0 g. NaIO₄; 10 g. K₂CO₃; 1.0 g. KMnO₄ in 500 ml. of distilled water (4)	sugar moieties
2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in benzene (5)	sulfides, sulfoxides, sulfones
0.5% ninhydrin in acetone	secondary amine in N-demethylclindamycin

Radioactive zones were located by scanning in a glass plate scanner[7].

Quantitative TLC was used to measure the relative amount of each component in the urine. Twenty-milliliter aliquots of urine
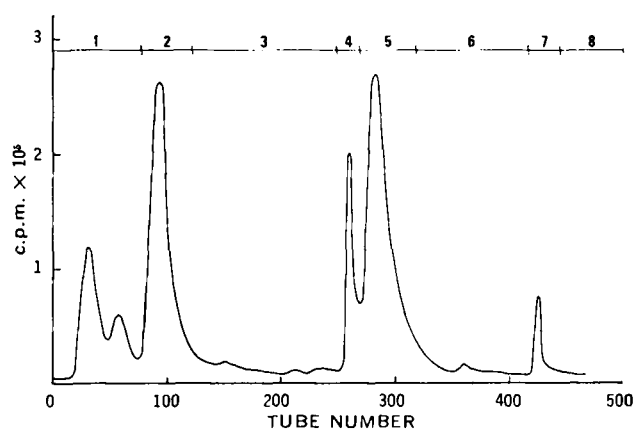

Figure 3—*Silica gel column chromatography of crude dog urine extract.*

| | Relative Amounts in Total Urine, % of Urinary Radioactivity | | | | | | | |
| | Radioactive Urine Collected the 1st Day | | | | Radioactive Urine Collected the Last Day | | | |
	1[b]	2[b]	3[c]	Mean	1[b]	2[b]	Mean	Average
Unchanged clindamycin	54.59	52.47	50.07	52.38	52.11	57.58	54.85	53.36 ± 2.55
Clindamycin sulfoxide	30.41	30.54	36.34	32.43	31.56	28.20	29.88	31.41 ± 2.7
N-Demethyl-clindamycin	14.95	16.97	13.50	15.14	16.2	13.82	15.04	15.10 ± 1.35

[a] Radioactive urine samples are collected from 0 to 48 hr. postadministration. [b] The solvent system used was chloroform–methanol (6:1). [c] The solvent system used was ethyl acetate–acetone–water (8:5:1).

samples collected from the clindamycin-treated rats or dogs were concentrated to 0.5 ml. The pH was adjusted to approximately 10 before 20 μl. of each was streaked on a 2.5 × 18-cm. thin-layer plate (250 μ thick), and the plate was developed with the TLC Solvent Systems c and e. After the development was completed, the silica gel was scraped in 1-cm. sections into 18 scintillation counting vials containing 0.5 ml. of water and 15 ml. of counting solvent[8] (1). The radioactivity was counted in a liquid scintillation counter[9]. Quenching losses were corrected by adding 0.05 ml. of tritiated toluene and counting again.

Enzymatic Analysis (Identification of Clindamycin Glucuronide)—The identity of clindamycin glucuronide was established by treatment with β-glucuronidase. Clindamycin glucuronide (20 mg.) was dissolved in 10 ml. of 0.1 M sodium acetate buffer (pH 5.0) and mixed with 0.1 ml. of concentrated β-glucuronidase aryl sulfatase solution[10]. The mixture was incubated in a 37° water bath for 24 hr. After the incubation period, the mixture was adjusted to pH 10.0 with 3.6 N potassium hydroxide and passed through a small (10 × 100 mm.) polystyrene resin column. The acetate buffer was washed off with 500 ml. of water. The clindamycin and clindamycin glucuronide were eluted with 200 ml. of 95% aqueous acetone and 200 ml. of methanol. The solvent eluates were pooled and concentrated under reduced pressure. Intact clindamycin (hydrolysis product) was isolated from the extract by preparative TLC in a solvent system composed of ethyl acetate acetone water (8:5:1). Identification of the hydrolytic product as intact clindamycin was based upon TLC behavior and mass spectrometry. As a control, another 20-mg. sample of clindamycin glucuronide was carried

through the entire procedure but without the addition of enzyme. The glucuronide was recovered unaltered.

For use as a chromatographic standard, authentic clindamycin glucuronide was synthesized enzymatically by reacting clindamycin with uridine diphosphoglucuronic acid (ammonium salt) in the presence of freshly prepared dog liver microsomes. The reaction product was separated from the unreacted clindamycin by polystyrene resin and silica gel chromatography.

Isolation of In Vitro Metabolites of Clindamycin —Preliminary experiments were conducted to study the metabolism of clindamycin under *in vitro* conditions. Livers from eight Sprague–Dawley rats (four male and four female), with the average body weight of 200 g., and from two male beagle dogs were used. The rats were sacrificed by cervical dislocation, and the dogs were sacrificed by electrocution. Microsomes were prepared by the differential centrifugation procedure of Allmann *et al.* (6), except 0.25 M sucrose–0.01 M tromethamine hydrochloride solution was used for suspension instead of sucrose–mannitol A.

N-Demethylation and sulfoxidation reactions of clindamycin were carried out in open 25-ml. erlenmeyer flasks at 38° in a metabolic shaker[11]. The complete reaction medium contained per each milliliter: potassium phosphate buffer (pH 7.5), 100 μmoles; magnesium chloride, 5 μmoles; nicotinamide-adenine dinucleotide phosphate (NADP), 1 μmole; nicotinamide-adenine dinucleotide (NAD), 1 μmole; sodium isocitrate, 5 μmoles; isocitrate dehydrogenase, 0.1 mg.; microsomes, 3 5 mg.; and clindamycin, 5 μmoles. The mixture was incubated for 1 hr. under air.

Glucuronic acid conjugation of clindamycin was carried out in a 25-ml. erlenmeyer flask at 38° under nitrogen atmosphere. The reaction mixture consisted of the following components per each milliliter: tromethamine hydrochloride, 100 μmoles; uridine diphosphate glucose (UDPG), 133 μmoles; magnesium chloride, 4 μmoles; clindamycin, 2 μmoles; and microsomes, 2–4 mg. The mixture was incubated for 1 hr. before extraction.

After the incubation was complete, the reaction was terminated by addition of 0.05 ml. of 3 M trichloroacetic acid/ml. of the reaction mixture. The excess acid was immediately neutralized by the addition of 3 M KOH. The denatured protein was removed by centrifugation. The clear supernate was adjusted to pH 8.5 with 3 M KOH and loaded into a small polystyrene resin column (10 × 100 mm.). The column was washed with 200 ml. of distilled water. Clindamycin and its metabolites were eluted with 200 ml. of 95% aqueous acetone and 200 ml. of methanol. The solvents were pooled and concentrated under reduced pressure. Drug-related compounds in the extract were isolated by preparative TLC and identified as described previously.

Figure 4—*Quantitative thin-layer chromatogram of lyophilized pooled rat urine. The solvent system was chloroform–methanol (6:1).*

RESULTS AND DISCUSSION

Rats—Figure 4 shows the thin-layer chromatograms of two crude rat urine extracts from polystyrene resin column chromatography. The solid line shows the distribution pattern of urinary radioactivity from rats that received a single dose of clindamycin hydrochloride, and the dotted line shows the same pattern from rats after chronic administration of the drug for a month. In both cases, three radioactive spots were observed. After further separation with countercurrent distribution and silica gel column chromatography, the three compounds were characterized as follows: Zone 1,
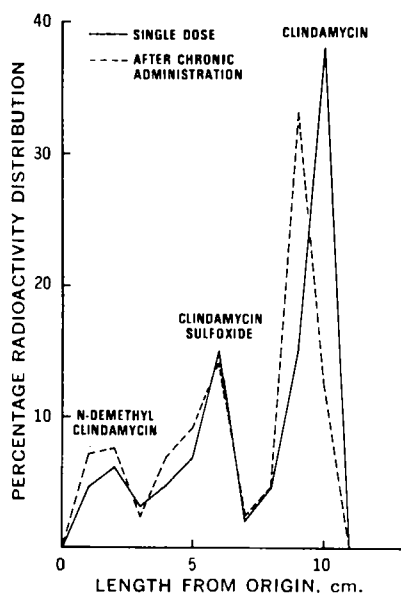
[8] Diotol.
[9] Packard Tri-Carb 314EX.
[10] Sigma Type H-2 β-glucuronidase, No. G-0876, 143,000 units/ml.

[11] Dubnoff.

unchanged clindamycin; Zone 2, clindamycin sulfoxide; and Zone 3, N-demethylclindamycin.

Appreciable quantities of both intact drug and clindamycin sulfoxide were isolated for an unequivocal identification. For the intact drug, results from all three spectroscopies and TLC were consistent with the authentic material.

For the clindamycin sulfoxide, mass spectrometry showed peaks (m/e) at 440 and 442, corresponding to the molecular ion; 377 and 379, due to the M—S(=O)CH₃ ion; 275 and 277, due to the M—methylthiogalactose ion; and 126, due to the propylhygric acid ion.

NMR spectroscopy showed that the protons of the S-methyl group were shifted downfield, which is consistent with the sulfoxide structure. IR (micro KBr pellet) and TLC results were identical to those for the reference material. Thus, the identity was established.

Since these spectra were presented elsewhere (3), they are not reproduced in this report.

The identity of N-demethylclindamycin was established mainly by TLC and mass spectrometry. The amount of isolated material was insufficient for a positive identification by NMR. This compound showed mass spectral peaks (m/e) at 410 and 412, corresponding to the molecular ion; 373 and 375, due to the M—SCH₃ ion; 261 and 263, due to the M—methylthiogalactose ion; and 112, due to the N-demethylpropylhygric acid ion. This was consistent with the assigned structure. Furthermore, the isolated material showed identical R_f values with an authentic sample of N-demethylclindamycin in all six TLC solvent systems tested. Both samples developed a purple color after spraying with ninhydrin reagent, which indicates the free secondary amine group. These results are all consistent with the identification of the unknown compound as N-demethylclindamycin.

Quantitative determination of the percentage of each component in rat urine was carried out with TLC (Fig. 4 and Table II). Similar results were obtained from five separate determinations of two pooled urine samples from rats given single or chronic doses of clindamycin with two different developing systems. In all cases, it was found that approximately one-half of the radioactivity in rat urine was unchanged clindamycin (53.36 ± 2.70%) and N-demethylclindamycin (15.10 ± 1.35%). These determinations were carried out on pooled urine samples, so the standard deviation represented only the variation of the TLC assays.

To determine whether chronic administration of clindamycin to rats would stimulate or inhibit its own metabolism, radioactive drug was given to these rats on the first and the last days of the 1-month dosing period, with unlabeled drug in between. The percentage of each major component in the two radioactive urine samples was measured and compared. The results (Table II) clearly revealed that the percentage of metabolites and unchanged drug did not change after chronic administration, which ruled out the possibility that clindamycin modified its own metabolism.

Dogs—Typical thin-layer chromatograms (Fig. 2) of the urine extracted from polystyrene resin column chromatography show three major radioactive zones plus one minor zone. Resolution of the same extract by silica gel column chromatography yielded eight fractions (Fig. 3 and Table I). Each fraction was characterized as follows.

Fraction 1—The materials under the two peaks were identical based upon TLC in three solvent systems. After purification by preparative TLC, this fraction yielded unchanged clindamycin as established by TLC, mass spectroscopy, and IR spectroscopy.

Fraction 2—This fraction was purified and identified as clindamycin sulfoxide by TLC and mass spectrometry.

Fraction 3—This fraction contained one radioactive compound, which appeared in the same R_f region as N-demethylclindamycin on the thin-layer chromatogram and also showed a positive ninhydrin reaction. It was tentatively identified as N-demethylclindamycin. Further confirmation was not possible since the quantity was too small.

Fractions 4 and 5—Both fractions were identical based upon TLC or countercurrent distribution. The isolated material was identified as clindamycin glucuronide by the following criteria:

1. Mass Spectroscopy—Low-resolution mass spectroscopy showed neither molecular ion nor any ion that contained a chlorine atom. Peaks were observed at m/e 388 (clindamycin free base — HCl), 345 (clindamycin free base — HCl — SCH₃), and 126 (hygric acid portion). It appeared that the compound lost hydrogen chloride readily upon electron impact.

High-resolution mass spectroscopy of the trimethylsilyl ether

Clindamycin + UDPGA + Dog Liver Microsomes*   **Clindamycin Glucuronide + β-Glucuronidase***   **Clindamycin Glucuronide Isolated from Dog Urine**
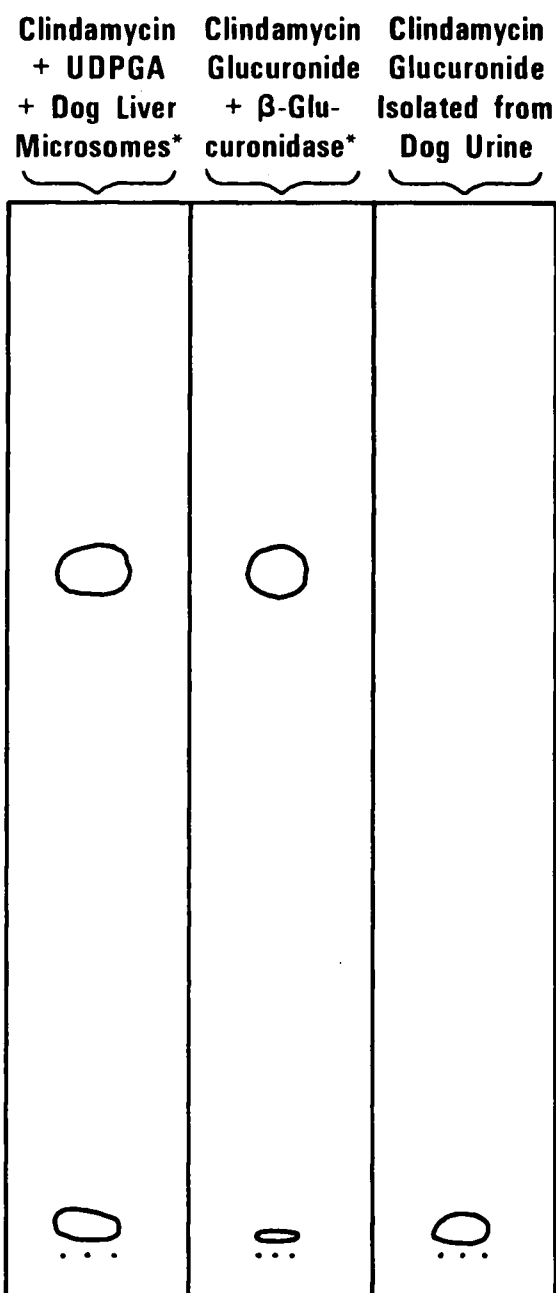
Figure 5—Thin-layer chromatogram of three clindamycin glucuronide preparations. The solvent system was ethyl acetate-acetone-water (8:5:1). (* Extracts from polystyrene resin column chromatography.)

derivative showed peaks at m/e 1032 (molecular ion) which was consistent with the hexa(trimethylsilyl) clindamycin monoglucuronide. Peaks were also seen at m/e 1017 (M—15): 966 (M—HCl); 989 (M—SCH₃); 375, due to the silylated sugar portion; and 126, due to the propylhygric acid portion of the molecule.

2. IR Spectroscopy—The IR spectrum (micro KBr) was consistent with a glucuronic acid derivative of clindamycin with an ether linkage.

3. NMR Spectroscopy—The spectrum was too complex for a detailed analysis. It was not possible to locate which hydroxyl group was conjugated with glucuronic acid on the S-methylthiogalactose.

4. Glucuronidase Treatment —After treatment with β-glucuronidase, the glucuronic acid was removed and unchanged clindamycin was regenerated. The reaction product was identified by both TLC and mass spectroscopy.

5. TLC—The thin-layer chromatograms of clindamycin glucuronide isolated from dog urine compared to authentic clindamycin

Table III—Metabolites Produced by Incubating Liver Microsomes with Clindamycin-^{3}H in the Presence of NADPH and O_2 for 30 min.

Enzyme Source	Clindamycin Sulfoxide Produced, mole^{-8}/mg.	Formaldehyde Produced, mole ^{9}/mg.	N-Demethyl-clindamycin Produced, mole^{-8}/mg.
Rat liver microsomes	28.25	45.81	6.15
Boiled rat liver microsomes	1.82	—	—
Dog liver microsomes	21.24	22.50	Not detectable
Boiled dog liver microsomes	2.97	-	—

glucuronide are shown in Fig. 5. The identical R_f values serve as further confirmation of their identity.

Fractions 7 and 8—The two fractions contained less than 2% of the total radioactivity placed on the column. TLC showed that both fractions were heavily contaminated by known drug-related components, *i.e.*, unchanged clindamycin, clindamycin sulfoxide, and clindamycin glucuronide. These compounds were probably the sources of radioactivity. Both fractions were discarded.

Quantitative determination of the percentage of each component in dog urine was carried out by TLC. An average of 35.69% of the total radioactivity excreted in urine was unchanged drug. The remaining consisted of 27.57% clindamycin sulfoxide, 27.58% clindamycin glucuronide, and 9.16% N-demethylclindamycin.

Metabolism of Clindamycin-^{3}H In Vitro—Preliminary evidence was obtained that liver microsomes from both species are capable of catalyzing the bioconversion of clindamycin to its metabolites (Table III). When radioactive clindamycin was incubated with freshly isolated rat or dog liver microsomes in the presence of NADPH and oxygen, the predominant product was clindamycin sulfoxide. A small but detectable amount of N-demethylclindamycin could also be observed. However, if semicarbazide was added to the reaction mixture, the N-demethylation reaction could be readily demonstrated by following the formaldehyde generated with Nash's (7) reagent. Both metabolic activities were abolished when boiled microsomes were used or NADPH was deleted from the reaction mixture.

Glucuronic acid conjugation could also be demonstrated, under *in vitro* conditions, by the liver microsomes from both species. Radioactive clindamycin was incubated with microsomes in the presence of uridine diphosphoglucuronic acid and magnesium salt at 37° for 1 hr. The reaction mixture was deproteinized and passed through a polystyrene resin column. Clindamycin glucuronide in very small quantity was isolated from the extract by preparative TLC. It had the same spectral and chromatographic properties as the material isolated from urine and could be converted back to clindamycin by treatment with β-glucuronidase. Since the conditions of the reaction have not been optimized, the results cannot be expressed quantitatively at present.

The study described here has established that clindamycin is metabolized by the rat through two well-known mechanisms: S-oxygenation and N-demethylation. On the other hand, S-oxygenation and glucuronic acid conjugation are the major metabolic

routes in the dog. However, since the major excretion route of clindamycin in both species is the feces (60–70%) rather than the urine, it would be risky to assume that the urinary composition of drug and metabolites reflects the overall picture.

The bioactivities of both clindamycin sulfoxide and N-demethylclindamycin have been determined in the past (3). N-Demethylclindamycin was approximately four times as effective as clindamycin in the antibacterial tests, whereas clindamycin sulfoxide has only one-quarter the activity of the parent drug.

The bioactivity of clindamycin glucuronide was determined[12], and the conjugate had practically no antibacterial activity (less than 0.02% of the antibacterial activity of clindamycin).

The species difference in glucuronic acid conjugation of clindamycin between rat and dog was quite interesting. The difference was probably partially due to a relative deficiency of uridine diphosphate-glucuronyltransferase in the rats used for this study. Investigation of the uridine diphosphate-glucuronyltransferase system in rat and dog liver showed that dog liver possessed five times more glucuronide synthesis activity than the rat liver using p-nitrophenol as substrate. A survey of the literature also indicated that rats possess the least glucuronide synthesis activity of the six species studied, *i.e.*, rabbit, guinea pig, mouse, sheep, and dog (8–10). However, since the metabolite profile of clindamycin in the feces was not known and the bioconversion of clindamycin to clindamycin glucuronide by rat liver microsomes could not be demonstrated, other factors, such as excretion through the bile, could be the cause for the failure to detect clindamycin glucuronide in rat urine.

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