Enhanced Rectal Absorption of Cefmetazole and Cefoxitin in the Presence of Epinephrine Metabolites in Rats and a High-Performance Liquid Chromatographic Assay for Cephamycin Antibiotics

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Abstract □ 4-Hydroxy-3-methoxymandelic acid and 3,4-dihydroxymandelic acid were found to be potent adjuvants for the rectal absorption of water-soluble compounds in rats. Both adjuvants enhanced the absorption of two cephamycin antibiotics, cefmetazole and cefoxitin. Maximum plasma levels of the antibiotics were obtained within 30 min after rectal administration. The bioavailability of both antibiotics appeared to depend on the concentration of the adjuvant in the microenema, the dosage form used in these experiments. Instead of a microbial assay, a new chemical method involving high-performance liquid chromatography with an ion-pairing technique was developed for analyzing the cephamycin antibiotic plasma levels.

Keyphrases □ Epinephrine metabolites—4-hydroxy-3-methoxymandelic acid, 3,4-dihydroxymandelic acid, adjuvants, rectal absorption of cefmetazole and cefoxitin, rats □ Cefmetazole—rectal absorption in rats, use of epinephrine metabolites as adjuvants, high-performance liquid chromatographic assay in plasma □ Cefoxitin—rectal absorption in rats, use of epinephrine metabolites as adjuvants, high-performance liquid chromatographic assay in plasma

Cefmetazole and cefoxitin, cephamycin antibiotics which are effective against Gram-positive and Gram-negative bacteria, are differentiated from cephalosporins by a methoxy group at the 7-position of the isoxazole ring. Both antibiotic agents are currently administered by intravenous or intramuscular injection.

In a number of situations, rectal administration of these drugs would be advantageous, especially to reduce the muscle damage caused by repeated injections. Previous work (1–5) demonstrated that salicylates and several structurally related compounds (*e.g.*, sodium 5-methoxysalicylate) markedly enhanced the rectal absorption of many types of drugs, particularly those that are soluble in water, including cefmetazole. Also, a relatively high concentration (50 mg/mL) of salicylate did not appear to cause damage to the rectal mucosal membrane (3).

This paper describes the effect of two metabolites of epinephrine, 4-hydroxy-3-methoxymandelic acid (I) and 3,4-dihydroxymandelic acid (II), on the rectal absorption of sodium cefoxitin and sodium cefmetazole in rats. The rectal absorption of phenolsulfonphthalein, used as a model water-soluble compound, was also studied.

Often, microbial assays such as that reported by Simon and Yin (6) have been used to measure plasma concentrations of antibiotics such as cefmetazole and cefoxitin. Buhs *et al.* (7) have reported a high-performance liquid chromatographic (HPLC) assay of cefoxitin using a strong anion-exchange resin. In this paper, we describe HPLC assays (using reverse-phase columns) for cefmetazole and cefoxitin that were developed to avoid the interference of additives administered with the antibiotics.

EXPERIMENTAL

Materials—A pump[1] equipped with an injector[2], a reverse-phase column[3], and a UV detector[4] (254 nm), all operated at room temperature, was used for analysis of cefoxitin and cefmetazole. The compounds used included sodium cefoxitin[5], sodium cefmetazole[6], 4-hydroxy-3-methoxymandelic acid[7], 3,4-dihydroxymandelic acid[7], acetonitrile[8], citric acid[9], sodium citrate[9], tetrabutylammonium bromide[9], and phenolsulfonphthalein[9].

Sample Preparation—Plasma aliquots of 1.0 mL were mixed with 0.1 mL of 0.01 M phosphoric acid and 0.6 mL of acetonitrile in 15-mL test tubes for 20–30 s and then centrifuged at ~2000 rpm for 5 min. The supernatants were poured into 4-mL glass vials and evaporated to dryness under a nitrogen stream using evaporating manifolds[10]. The residues were dissolved in 0.1 mL of water just before analysis and stirred vigorously using a vortex mixer. Sample aliquots of 20 μL were then chromatographed. Blank plasma samples spiked with drug concentrations of up to 3.0 μg/mL were also analyzed to establish a calibration curve.

Chromatographic Conditions—Two different reverse-phase columns were used, and appropriate conditions were developed for each. A mobile phase consisting of 8 volumes of 0.005 M citrate buffer, pH 5.0, containing 0.002 M tetrabutylammonium bromide (ion-pairing agent) and 2 volumes of acetonitrile was used for the Ultrasphere ODS column[3]. A mixture consisting of 77.5% (v/v) 0.025 M citrate buffer, pH 5.0, containing 0.005 M tetrabutylammonium bromide and 22.5% (v/v) acetonitrile was used as the mobile phase for the OD-MP, RP-18 column[3]. The flow rate of the mobile phase was 1.0 mL/min. The column effluent was monitored by UV absorption at 254 nm, and peak height measurements were used for quantitation. The column was maintained at room temperature.

Animals—Sprague–Dawley male rats, 225–250 g, were fasted for 16 h prior to the experiment to avoid residual feces in the rectum which could reduce drug absorption. The rats were anesthetized with pentobarbital (60 mg/kg), and the rats' body temperatures were maintained at 38°C using a temperature-controlled platform.

***In Vivo* Absorption Study**—The drugs and adjuvants were administered rectally as a 0.3- or 0.6-mL microenema using 0.01 M phosphate buffer at pH 4.5 as the vehicle. Following administration of the drug solution to the rat, the anus was ligated with a thread to avoid leakage of the solution. Blood samples were withdrawn from the jugular vein at regular intervals into syringes pretreated with 3% sodium citrate (as anticoagulant) and centrifuged at 3000 rpm for 10 min.

Assay for Phenolsulfonphthalein in Plasma—Plasma samples of 0.3 mL were diluted with 0.3 mL of 0.1 M NaOH solution and measured with a spectrophotometer at 540 nm. As a blank, 0.3 mL of plasma was collected before administration of the phenolsulfonphthalein microenema.

RESULTS AND DISCUSSION

Drug Analysis—Due to the high polarity of cefoxitin and cefmetazole,

[1] Model 110A, Altex.
[2] Model 7010, Rheodyne.
[3] Ultrasphere ODS, 5 μm, 4.6 i.d. × 15-cm length; OD-MP, RP-18, 5 μm, 4.6 i.d. × 15-cm length; Altex.
[4] Model 153, Altex.
[5] Merck, Rahway, N.J.
[6] Sankyo Co., Tokyo, Japan.
[7] Sigma Chemical Co., St. Louis, Mo.
[8] Fisher Chemical Co., St. Louis, Mo.
[9] Aldrich Chemical Co., Milwaukee, Wis.
[10] Silli-Vap Evaporator; Pierce Chemical Co., Rockford, Ill.

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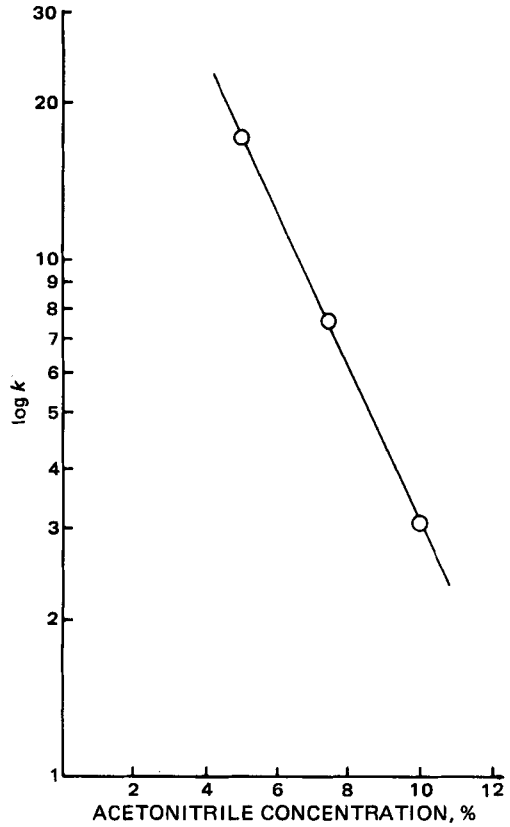

Figure 1—*Relationship between the capacity factor (k′) of cefmetazole and concentration of acetonitrile in the mobile phase. Chromatographic conditions: Ultrasphere ODS (4.6-mm × 15-cm) column with a mobile phase of 0.005 M citrate buffer (pH 5.0)–acetonitrile at a flow rate of 1 mL/min and detection at 254 nm.*

an aqueous or highly polar mobile phase with a high resolution reverse-phase column was used for the analysis. Reverse-phase chromatography using a mixture of buffer and acetonitrile as the mobile phase was the first analytical method attempted for the determination of cefmetazole and cefoxitin. With this method, the relationship between the percent concentration of acetonitrile in the mobile phase *versus* the capacity factor (log k') proved to be linear (Fig. 1). Optimum conditions for this method

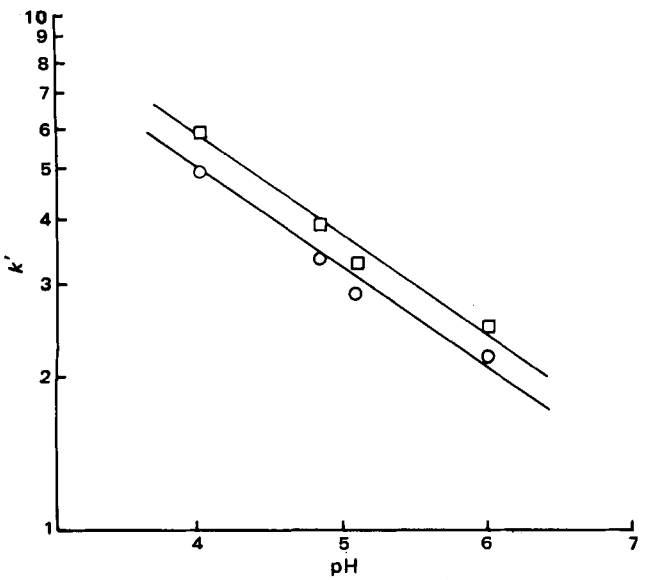

Figure 2—*Ion-pair reverse-phase chromatographic separation of cefmetazole (○) and cefoxitin (□) as a function of pH. Chromatographic conditions: Ultrasphere ODS (4.6-mm × 15-cm) column with a mobile phase of 0.002 M tetrabutylammonium bromide in 0.005 M citrate buffer–acetonitrile (8:2).*

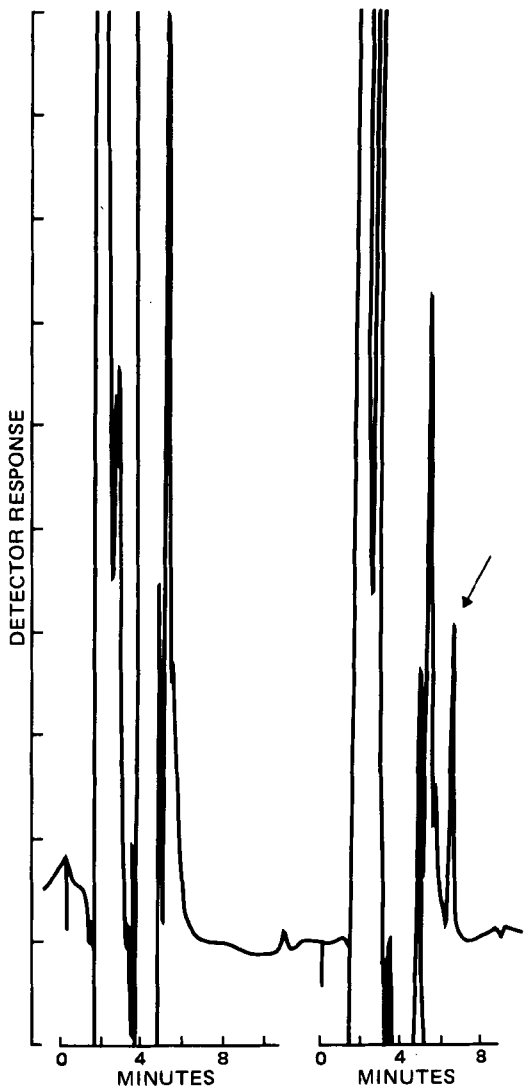

Figure 3—*Chromatogram of cefmetazole in plasma using an Ultrasphere ODS (4.6-mm × 15-cm) column with a mobile phase of 0.002 M tetrabutylammonium bromide in 0.005 M nitrate buffer (pH 5.0)–acetonitrile (8:2). The arrow indicates the cefmetazole peak, and the number of AUFS is 0.01.*

were obtained when the acetonitrile concentration was between 8 and 10% and the capacity factor was the only factor under consideration. It was critical that the retention time in each mobile phase be the same; however, using this method, some of the other plasma components interfered with the cefmetazole peak. Therefore, an ion-pairing agent (tetrabutylammonium bromide) was added to the mobile phase to enhance the retention of the drug.

Theoretically in reverse-phase ion-pairing chromatography, maximum k' values are obtained at intermediate pH levels, where the sample compounds are completely ionized and ion-pair formation is at a maximum. Since the pK_a values of cefoxitin and cefmetazole are 2.50 and 2.34, respectively, as the pH of the mobile phase increases, the k' values of these drugs should also increase if they are retained as ion-pair complexes. However, in our experiments, cefmetazole and cefoxitin appeared to be retained to a greater extent as the pH of the mobile phase decreased. The effect of pH on the separation of the components using tetrabutylammonium bromide as the source of the counter ion is shown in Fig. 2. One possible explanation for the results obtained may be that ion pairs were formed between the drugs and tetrabutylammonium bromide which increased the retention time possibly by modifying the properties of the stationary phase.

Figure 3 shows a typical chromatogram for the assay of cefmetazole in plasma using an Ultrasphere ODS column. Cefoxitin has a slightly longer retention time, as shown in Fig. 2. Adjuvants such as 4-hydroxy-3-methoxymandelic acid or 3,4-dihydroxymandelic acid present in the plasma samples did not interfere with the drug assay procedure.

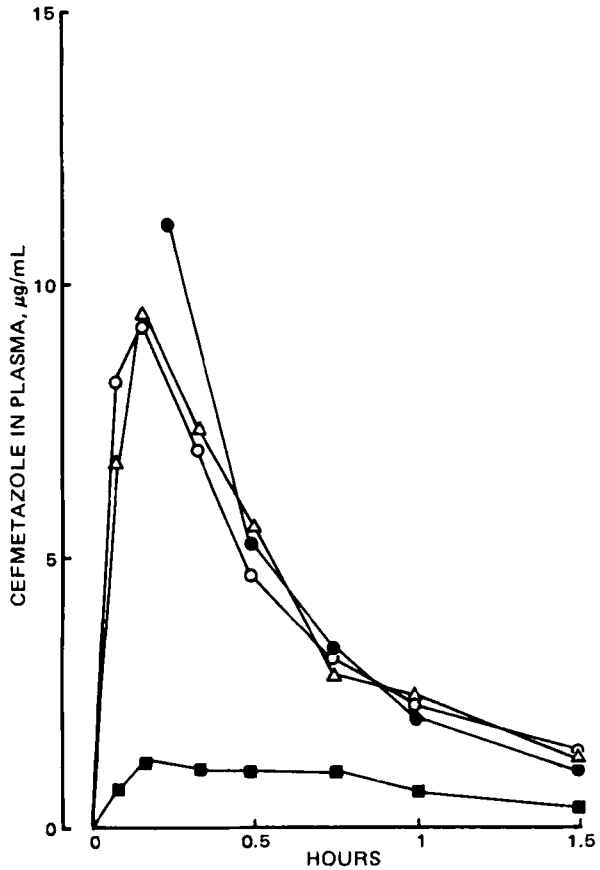

Figure 4—*Plasma cefmetazole levels in rats after intravenous injection of 6 mg/kg (●) and after rectal administration of a microenema containing 30 mg/mL/kg and 10 mg of sodium chloride (■); 30 mg/mL/kg, 10 mg of sodium chloride/mL, and 30 mg of I/mL/kg (○); or 30 mg/mL/kg, 10 mg of sodium chloride/mL, and 30 mg of II/mL/kg (△).*

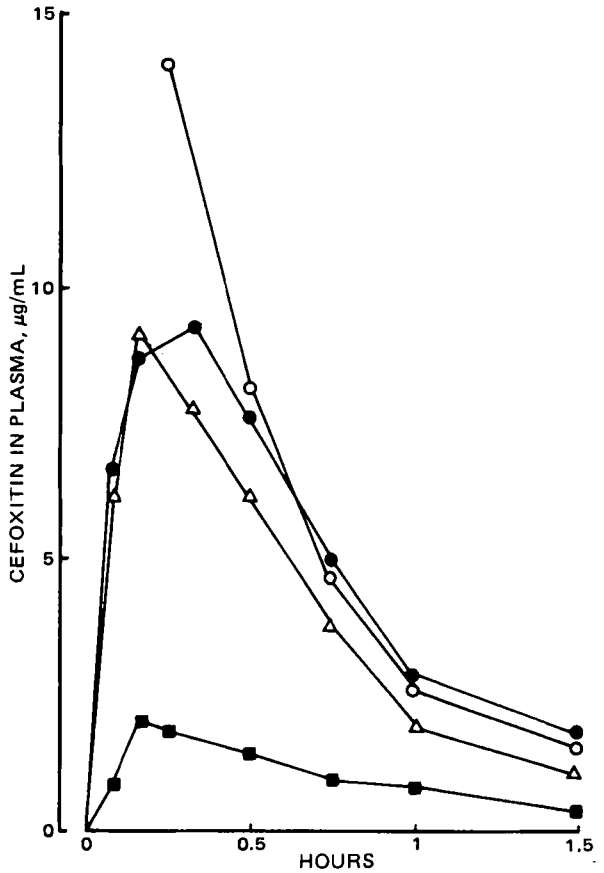

Figure 5—*Plasma cefoxitin levels in rats after intravenous injection of 6 mg/mL/kg (○) and after rectal administration of a microenema containing 30 mg/mL/kg and 10 mg of sodium chloride (■); 30 mg/mL/kg, 10 mg of sodium chloride, and 30 mg of I/mL/kg (●); or 30 mg/mL/kg, 10 mg of sodium chloride, and 30 mg of II/mL/kg (△).*

Linear calibration curves for cefoxitin and cefmetazole in water and plasma were obtained with concentrations ranging from 0.1 to 3.0 μg/mL. The reproducibility was indicated by coefficients of variation of 3.2% for cefmetazole and 1.5% for cefoxitin or five replicate analyses of 1.0-μg/mL samples. The recoveries of cefoxitin and cefmetazole, which were tested using plasma blanks containing the standard solution, were 91 and 90%, respectively. The limit for accurate quantitation appeared to be as low as 0.1 μg/mL for both compounds. When using columns from different manufacturers, minor modifications were necessary for good separation. Using OD-MP, RP-18, for example, the mobile phase described in the *Experimental* section was developed in order to get a separation comparable to the one shown in Fig. 3 with an Ultrasphere ODS column.

Table I—Relative Bioavailability of Cefmetazole (30 mg/kg) or Cefoxitin (30 mg/kg) after Rectal Administration in the Presence or Absence of Adjuvant

Adjuvant, mg/kg	Relative Bioavailability[a]	
	Cefmetazole	Cefoxitin
4-Hydroxy-3-methoxymandelic acid		
0	1.0	1.0
15	3.8 ± 1.3	2.6 ± 0.9
30	6.8 ± 2.5	5.7 ± 1.4
50	10.4 ± 2.8	9.3 ± 2.7
75	13.7 ± 3.4	15.6 ± 4.3
100	15.8 ± 3.1	17.5 ± 3.8
3,4-Dihydroxymandelic acid		
0	1.0	1.0
15	3.7 ± 0.8	2.9 ± 1.1
30	7.1 ± 2.1	4.8 ± 0.8
50	11.1 ± 2.3	7.4 ± 2.1
75	16.4 ± 4.2	14.2 ± 3.9
100	18.2 ± 4.8	19.3 ± 4.4

[a] Relative bioavailability = $([AUC]_{R \text{ in presence of adjuvant}}) \times (Dose_{\text{without adjuvant}}) / ([AUC]_{R \text{ without adjuvant}}) \times (Dose_{\text{in presence of adjuvant}})$.

Effects of Adjuvants—In a recent paper (5) describing the effects of several adjuvants on the rectal absorption of theophylline, lidocaine, cefmetazole, and levodopa, it was suggested that compounds which were effective in enhancing rectal absorption contained both hydroxy and carboxy functional groups. In these cases the groups were attached to a phenyl ring. To further elucidate the structural requirements for effective adjuvants, the present study involves two metabolites of epinephrine, 4-hydroxy-3-methoxymandelic acid (I) and 3,4-dihydroxymandelic acid (II), in which the carboxyl groups are not attached to a phenyl ring.

As seen in Figs. 4 and 5, respectively, these adjuvants improved the rectal absorption of cefmetazole and cefoxitin. Their action appeared to parallel that of 5-methoxysalicylate and other adjuvants described previously (5), in which relatively rapid absorption with high plasma levels was attained. Maximum plasma levels of cefmetazole appeared within 30 min after rectal administration with I or II. Higher plasma cefoxitin or cefmetazole levels were obtained after rectal administration of a microenema containing an adjuvant compared with the rectal administration of cefoxitin or cefmetazole alone (Figs. 4 and 5). As summarized in Table I, the relative bioavailabilities of cefmetazole and cefoxitin in the presence of adjuvants were significantly improved as determined from the typical relationship: $([AUC]_{R \text{ in presence of adjuvant}}) \times (Dose_{\text{without adjuvant}}) / ([AUC]_{R \text{ without adjuvant}}) \times (Dose_{\text{with adjuvant}})$.

The influence of adjuvant concentration is also illustrated in Table I. When the concentration of the adjuvant and drug in the microenema were cut in half by administering the same total amounts in double the solution volume, the cefmetazole plasma level decreased (Fig. 6). Therefore, the concentration of adjuvant and drug in the microenema appear to be important with regard to the efficacy of adjuvant-enhanced absorption.

Phenolsulfonphthalein has been used as an indicator to measure the change of perfusate volume in an *in situ* perfusion method since it is not readily absorbed (8). In this study, phenolsulfonphthalein was used as a typical water-soluble compound, *i.e.*, poorly absorbed from the rectum. In the absence of an adjuvant in the microenema, no phenolsulfonphthalein appeared in the plasma after rectal administration. However,

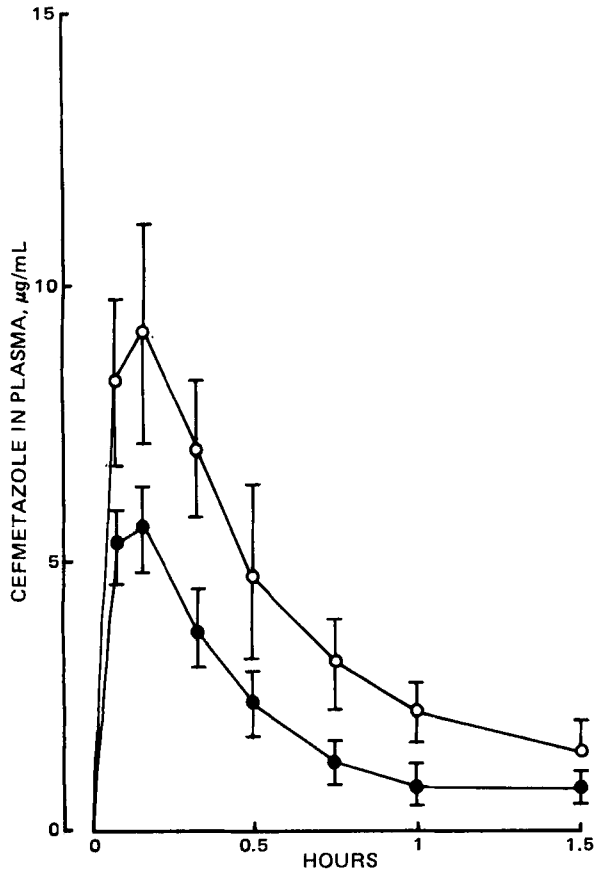

Figure 6—*Plasma cefmetazole levels in rats after rectal administration of a microenema containing 30 mg/mL/kg, 10 mg of sodium chloride, and 30 mg of I/mL/kg (○) or 30 mg/2 mL/kg, 10 mg of sodium chloride, and 30 mg of I/2 mL/kg (●). Statistical significance:* $p < 0.001$ ○ versus ● *except for 1.5 h.*

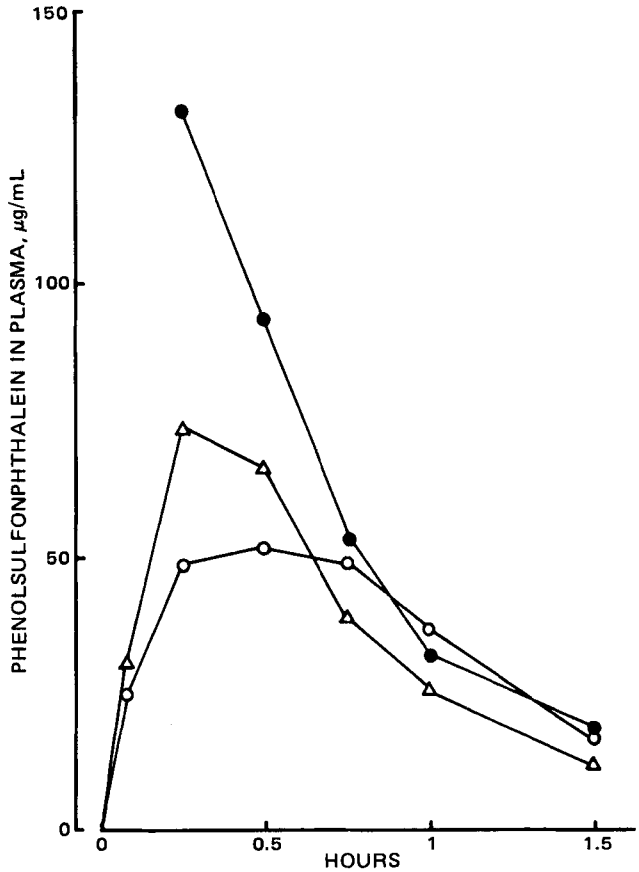

Figure 7—*Plasma phenolsulfonphthalein levels in rats after intravenous injection of 10 mg/kg and after rectal administration of a microenema containing 20 mg/mL/kg, 10 mg of sodium chloride, and 30 mg of I/mL/kg (○) or 20 mg/mL/kg, 10 mg of sodium chloride, and 30 mg of II/mL/kg (△).*

as shown in Fig. 7, absorption of phenolsulfonphthalein from the rectum was significantly enhanced in the presence of I or II. Bioavailability of phenolsulfonphthalein after rectal administration compared with intravenous injection was 32.4 ± 9.5% in the presence of 30 mg of I and 26.8 ± 5.7% in the presence of 30 mg of II.

The complete mechanism for the enhancement of rectal drug absorption in the presence of I and II has not been elucidated. It was reported earlier (1) that the enhancing action of salicylate was suppressed by the presence of the divalent ions of calcium and magnesium. Salicylate is known to complex with divalent metal ions, and it has been suggested that such interactions are responsible for at least some of the pharmacological and biochemical effects of salicylate (9). The presence of 10 mg/mL of calcium chloride in microenemas containing 30 mg/kg of cefoxitin or cefmetazole and 30 mg/kg of either I or II, reduced the peak plasma drug levels by ~50% compared with the absence of calcium chloride; *i.e.*, the presence of calcium chloride reduced the peak plasma levels of cefmetazole and cefoxitin from 9 μg/mL to 4 μg/mL in the presence of I and from 9 μg/mL to 5 μg/mL in the presence of II. Thus, it appears that the effects of I and II are similar to that of salicylate and that the adjuvants may require some complexation with divalent metal ions.

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