# Effect of Salicylate on the Rectal Absorption of Lidocaine, Levodopa, and Cefmetazole in Rats

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**Abstract** □ Salicylic acid and sodium salicylate have been found to enhance the absorption of lidocaine, levodopa, and cefmetazole after rectal administration to rats. These drugs represent a base (lidocaine), an acid (cefmetazole), and a substance (levodopa) which exists as a zwitterion in solution. The rectal absorption of each type of drug, as well as theophylline, a neutral compound, was enhanced by salicylate, particularly at pH values where the substances exist primarily in their ionic form. A requirement for the observed enhancement is that salicylate be present in the rectal membrane. The loss of drug from the perfusing solution was greater from solutions having an ionic strength of 0.75 than from those with $\mu = 0.15$.

**Keyphrases** □ Absorption, rectal—effect of salicylate on rectal absorption of lidocaine, levodopa, and cefmetazole, rats □ Salicylate—effect on rectal absorption of lidocaine, levodopa, and cefmetazole, rats □ Lidocaine—effect of salicylate on rectal absorption, rats □ Levodopa—effect of salicylate on rectal absorption, rats □ Cefmetazole—effect of salicylate on rectal absorption, rats

It has been reported (1, 2) that salicylate markedly enhanced the rectal absorption of theophylline. It was suggested that the effect of salicylate depends on its presence in the rectal membrane. It was also observed that the changes in absorption required the presence of salicylate in the formulation and that the mechanism of enhancement is different from that of surfactants.

The present report describes the extension of the use of salicylate to enhance the absorption of lidocaine, levodopa, and cefmetazole after rectal administration to rats. The drugs selected represent four different chemical classes: theophylline is a neutral substance, lidocaine is a basic material, cefmetazole is acidic, and levodopa exists as a zwitterion in solution. Furthermore, each of these drugs exhibits some difficulties in its administration and absorption. For example, it has been reported (3) that levodopa given rectally resulted in no rise in blood levodopa concentrations and no clinical benefit after rectal administration to parkinsonian patients. The purpose of this study was to examine to what extent the effects of salicylate are general and the extent rectal absorption enhancement depends on the specific drugs used. In addition, studies involving intravenous administration of salicylate were included to provide additional information about the mechanism of enhancement.

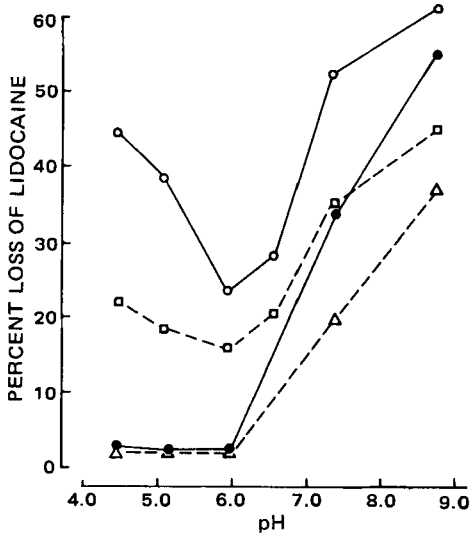


**Figure 1**—*Effect of pH, ionic strength, and salicylate on the disappearance of lidocaine hydrochloride from a perfusate in the rat rectum after 1 hr. The initial lidocaine hydrochloride concentration was 500 μg/ml, and the sodium salicylate concentration was 0.5 (○ and □) or 0% (● and △). The ionic strength was 0.75 (—) or 0.15 (- - -).*

## EXPERIMENTAL

**Materials**—Sodium salicylate[1], lidocaine[2], sodium cefmetazole[3], and levodopa[3] were used as obtained from the manufacturers.

**Animals**—Sprague-Dawley male rats (200–300 g) were used in this study and were fasted for 16 hr prior to the experiment. During the experiment, the rats were kept on a 38° surface and were anesthetized with pentobarbital (60 mg/kg).

The *in situ* perfusion of the rat rectum and the *in vivo* absorption studies were carried out as described previously (1, 2) with the following modifications: The pH of the perfusate was maintained constant by either the addition of 0.1 *N* NaOH or 0.1 *N* HCl as needed. Some experiments were also carried out at an ionic strength of 0.15 and 0.75 using sodium chloride to obtain the desired ionic strength. The *in vivo* absorption studies involved administering 0.3 ml of the drug solution into a 2-cm section of the rectum which was isolated by ligation with thread.

**Intravenous Infusion Studies**—To maintain constant plasma salicylate concentrations, an intravenous infusion method was used for some experiments. After the rats were anesthetized with pentobarbital, a sodium salicylate solution (300 mg/ml), adjusted to pH 7.4 with 0.0114 *M* phosphate buffer, was infused into the leg vein of the rat through a polyethylene canula at a flow rate of 0.05 ml/min during the first 10 min and then at a flow rate of 0.02 ml/min for another 60 min. Beginning 10 min after starting the infusion of the salicylate solution, blood samples

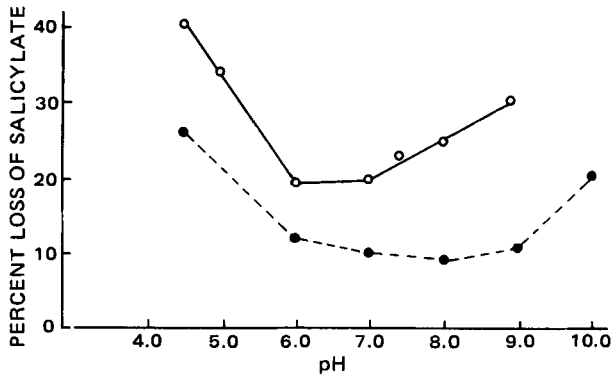


**Figure 2**—*The percent loss of salicylate from a perfusate as a function of pH having an initial concentration of 0.5% and ionic strength of 0.75 (○) and 0.15 (●).*

[1] Aldrich Chemical Co., 99+%.
[2] Sigma Chemical Co.
[3] Sankyo.

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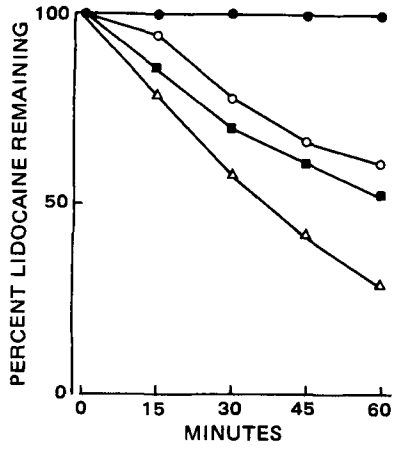


**Figure 3**—*The percent of lidocaine hydrochloride remaining in perfusate at pH 4.5 and an ionic strength of 0.75 with an initial lidocaine hydrochloride concentration of 500 μg/ml and sodium salicylate concentrations of 0 (●), 0.3 (○), 0.5 (■), and 1% (△).*

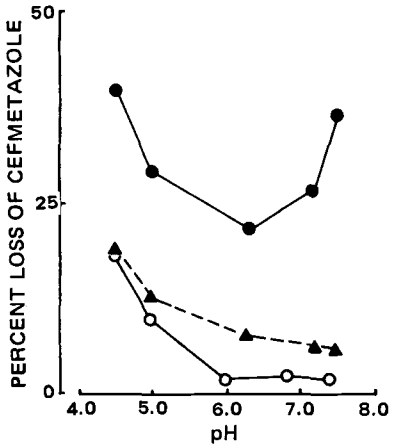


**Figure 4**—*Effect of pH and salicylate on the disappearance of cefmetazole from a perfusate in the rat rectum after 1 hr. The initial cefmetazole concentration was 200 μg/ml (○, ▲, and ●) and the sodium salicylate concentration was 0.5% (● and ▲). The ionic strength was 0.75 (○ and ●) and 0.15 (▲).*

were taken from the jugular vein at regular intervals to measure the concentration of salicylate in the plasma.

**Assay Methods**—Salicylate, theophylline, lidocaine, and levodopa were assayed by HPLC as described previously (2). For lidocaine a strong cation exchange column of 25-cm length was used. The mobile phase was 0.1 *M* acetate buffer at pH 3.0. The flow rate was 1 ml/min and a UV detector at 220 nm was used. For levodopa a reversed-phase column with a water–methanol mixture (95:5) as mobile phase was employed. The system was monitored at 254 nm.

The salicylate in the plasma was extracted with ether at a pH <2.0 after deproteinization with a 3.0% trichloroacetic acid solution. Following centrifugation, the ether layer was evaporated and the sediment was dissolved in methanol.

The lidocaine in the plasma was extracted with ether at pH 9.0 after deproteinization with a 3.0% trichloroacetic acid solution. Following centrifugation, the ether layer was evaporated and the sediment was dissolved in methanol.

**Table I—Absolute Bioavailability of Cefmetazole as a Function of Initial Salicylate Concentration**

| Salicylate Dose, mg/kg | Bioavailability, % |
|---|---|
| 0 | — |
| 5 | 22.8 ± 6.5[a] |
| 10 | 34.5 ± 9.2 |
| 15 | 52.5 ± 7.3 |
| 25 | 91.4 ± 12.3 |
| 40 | 98.6 ± 15.7 |

[a] Uncertainties are expressed as standard deviations ($n \geq 4$).

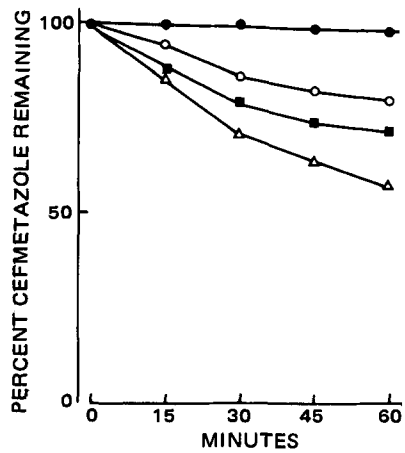


**Figure 5**—*The percent of cefmetazole remaining in perfusate at a pH of 7.4 and an ionic strength of 0.75 with an initial cefmetazole concentration of 200 μg/ml and sodium salicylate concentrations of 0 (●), 0.3 (○), 0.5 (■), and 1% (△).*

The levodopa in the plasma was extracted with acetone (5 times the sample volume). Following centrifugation, the supernate was evaporated and the sediment was dissolved in water.

Cefmetazole was also assayed by liquid chromatography using a high resolution reversed-phase column and an ion-pairing technique. The mobile phase consisted of a mixture of 77.5% (v/v) 0.025 *M* citrate buffer at pH 5.0 containing 0.005 *M* tetrabutylammonium (ion-pairing agent) and 22.5% (v/v) acetonitrile. 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.

One-milliliter cefmetazole plasma samples were mixed with 0.1 ml of 0.01 *M* phosphoric acid and 0.6 ml of acetonitrile for 20–30 sec and then centrifuged at ~2000 rpm for 5 min. The supernate was then evaporated to dryness under nitrogen and the residue dissolved in 0.1 ml of water just prior to assay. Blank plasma samples containing drug concentrations up to 30 μg/ml were also measured to establish a calibration curve.

## RESULTS AND DISCUSSION

**Drug Disappearance from Perfusate**—The pH profile of the effect of salicylate on the disappearance of lidocaine from a perfusate in the rat rectum is shown in Fig. 1. At pH values >6 and in the absence of salicylate, lidocaine disappeared readily from the perfusate and the presence of salicylate enhanced absorption only moderately. However, lidocaine loss was very small from acidic solutions in the absence of salicylate. At pH values <6, the presence of salicylate markedly enhanced the loss of lidocaine from perfusate solutions.

It is also apparent from Fig. 1 that the loss of lidocaine from the perfusate is greater with an ionic strength of 0.75 than at 0.15 using sodium chloride to adjust the ionic strength. As shown in Fig. 2, the loss of sali-

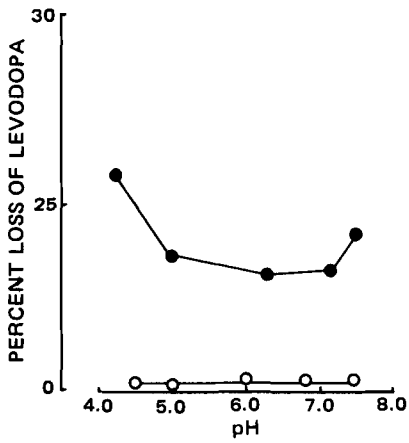


**Figure 6**—*Effect of pH and salicylate on the disappearance of levodopa from a perfusate in the rat rectum after 1 hr. The initial levodopa concentration was 200 μg/ml (○ and ●) and the sodium salicylate concentration was 0.5% (●).*

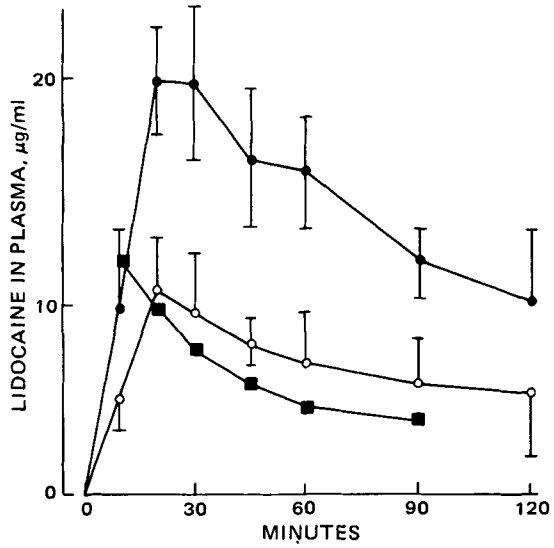


**Figure 7**—*Concentration of lidocaine in the plasma as a function of time after rectal administration of a microenema at pH 4.5 and a lidocaine hydrochloride dose of 60 mg/kg (● and ○) and sodium salicylate doses of 7.5 (○) and 15 mg/kg (●). Uncertainties are expressed as standard deviations by the error bars with* n = 6. *Lidocaine hydrochloride was also given intravenously (■) with a dose of 20 mg/kg.*

cylate is also greater at $\mu = 0.75$ than 0.15 from the perfusate at all pH values studied.

It appears that lidocaine is well absorbed from the rat rectum in the nonionic form which would predominate at basic pH values, but is not well absorbed from acidic solutions in which lidocaine exists as the protonated species. The presence of salicylate in the perfusate significantly increases the absorption of the water soluble form which is not absorbed effectively in the absence of salicylate.

As shown in Fig. 3, increasing the initial concentration of salicylate results in more rapid loss of lidocaine from the perfusate at pH 4.5. However, as reported previously (2), the disappearance rate constant of salicylate from the perfusate does not depend on the initial concentration of salicylate in the perfusate at a pH of 4.5, indicating that the disappearance of lidocaine from perfusate at a pH of 4.5 may depend on the amount of salicylate in the rectal membrane.

The loss of cefmetazole from perfusate in the rat rectum in the absence of salicylate was small as illustrated in Figs. 4 and 5. The addition of salicylate to the perfusate facilitated the disappearance of cefmetazole at all pH values. Again, the greatest enhancement was found at pH values at which the drug existed in the ionized state and at the higher ionic strength ($\mu = 0.75$). In fact, the effect of ionic strength is more pronounced for cefmetazole than for lidocaine. Furthermore, it was again observed that increasing concentrations of salicylate resulted in a more rapid disappearance of cefmetazole from the perfusing solution.

The presence of salicylate in the perfusate also promoted the disappearance of levodopa which was not absorbed significantly in the absence of salicylate at any pH (Fig. 6). The greatest enhancement occurred at

**Table II—The Effect of Salicylate Given Intravenously on the Loss of Cefmetazole and Theophylline from Perfusate** [a]

| Minutes | Plasma Salicylate Concentration, mg/ml | Percent Remaining in Perfusate | Percent Remaining with no Salicylate (intravenous) |
|---|---|---|---|
| Cefmetazole | | | |
| 0 | 1.99 ± 0.35 | 100 | 100 |
| 15 | 2.15 ± 0.28 | 100 | 100 |
| 30 | 2.28 ± 0.43 | 98.4 ± 0.5 | 99.5 ± 0.2 |
| 45 | 2.12 ± 0.30 | 97.6 ± 0.9 | 98.3 ± 0.8 |
| 60 | 2.03 ± 0.18 | 95.8 ± 1.2 | 96.2 ± 1.9 |
| Theophylline | | | |
| 0 | 2.13 ± 0.30 | 100 | 100 |
| 15 | 2.38 ± 0.43 | 100 | 100 |
| 30 | 2.45 ± 0.26 | 99.1 ± 0.3 | 99.2 ± 1.0 |
| 45 | 2.08 ± 0.25 | 97.8 ± 1.1 | 97.3 ± 0.8 |
| 60 | 2.21 ± 0.31 | 95.8 ± 1.1 | 95.4 ± 2.1 |

[a] Uncertainties are expressed as standard deviations ($n \geq 4$).

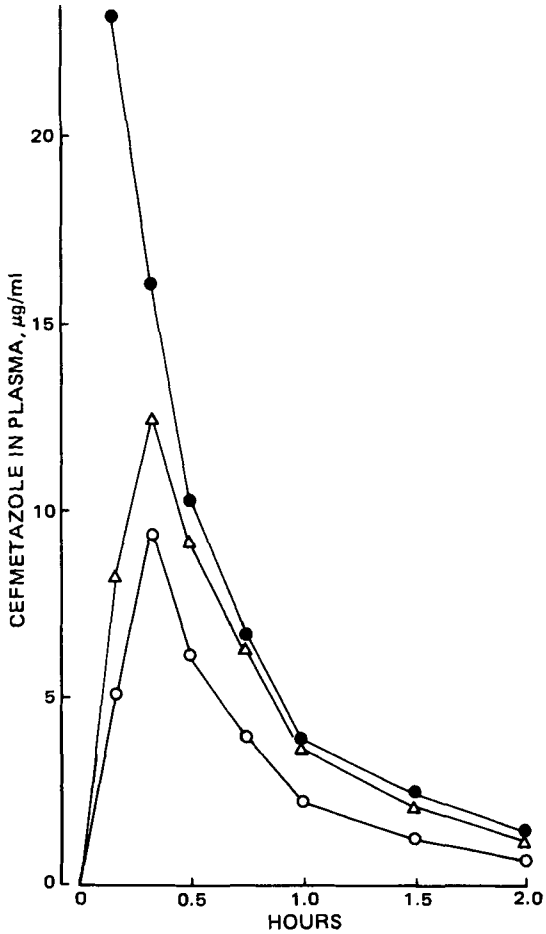


**Figure 8**—*Concentration of cefmetazole in the plasma as a function of time following rectal administration of a microenema at pH 7.4 and a cefmetazole dose of 15 mg/kg and 25 (○) or 40 mg (△) of sodium salicylate. Cefmetazole (10 mg/kg) was also given intravenously (●).*

pH values <5 and >7. Loss of levodopa was also more rapid with increasing concentrations of salicylate in the perfusate.

It was previously reported (1, 2) that salicylate itself was well absorbed at pH values <5 and >7.4. Thus, the disappearance rates of lidocaine, cefmetazole, and levodopa appear to depend on the effective disappearance of salicylate.

**Plasma Levels Obtained from Microenemas**—Lidocaine was administered as a solution at pH 4.5 because the perfusate studies indicated that it was absorbed at pH 7.4 in the absence of salicylate. Cefmetazole and levodopa were administered in solutions having pH values of 7.4.

As shown in Fig. 7, the plasma levels of lidocaine increased rapidly after rectal administration in the presence of salicylate. However, in the absence of salicylate, no lidocaine was found in the plasma. The detection limit for lidocaine was 2.5 μg/ml. Figure 6 also indicates that a higher dose of salicylate resulted in a higher blood level of lidocaine. As reported earlier (2), upon addition of salicylate at doses >15 mg/kg, the absolute bioavailability of lidocaine was nearly 100%.

Salicylate also markedly increased the blood levels of cefmetazole as illustrated in Fig. 8. Without the presence of salicylate, no cefmetazole was detected in the plasma. The minimum assay sensitivity was 0.1 μg/ml; however, in the presence of salicylate in solution, the plasma cefmetazole levels increased rapidly with maximum levels being reached 30 min after rectal administration. The absolute bioavailability as a function of salicylate concentration is shown in Table I.

As shown in Fig. 9, similar results were obtained for levodopa. In the absence of salicylate, levodopa was not found in the plasma. The sensitivity of the assay was 0.25 μg/ml. The absolute bioavailability of levodopa was ~75% in the presence of 15 mg of salicylate/kg.

These results indicate that salicylate may facilitate the rectal absorption of many types of drug substances, particularly in their ionic form.

**Requirement of Salicylate in the Rectal Membrane**—The disappearance of drug from the rectum appears to depend on the concurrent

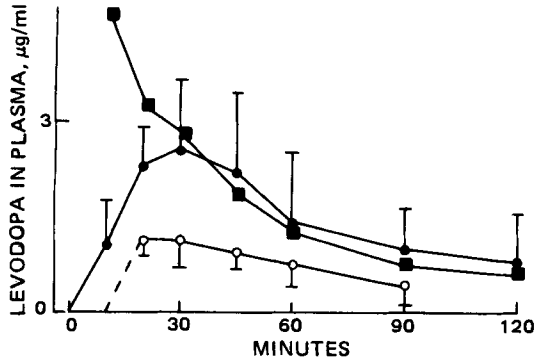


**Figure 9**—*Concentrations of levodopa in the plasma as a function of time following rectal administration of a microenema at pH 7.4 and a levodopa dose of 15 (● and ○), 7.5 (○), and 15 mg/kg (●) of sodium salicylate. Levodopa (10 mg/kg) was also given intravenously (■).*

rise in the plasma salicylate concentration after the simultaneous rectal administration of both salicylate and drug as shown in Fig. 10 for cefmetazole and levodopa. To examine the effect of salicylate levels independent of rectal absorption, the effect of salicylate given intravenously on the rectal absorption of cefmetazole and theophylline was studied. In this study, sodium salicylate was given by an intravenous infusion to maintain a relatively high plasma salicylate concentration of ~2 mg/ml. As shown in Table II and Fig. 10, salicylate in the plasma alone did not affect the loss of cefmetazole or theophylline from the solution perfusing the rat rectum. Furthermore, no salicylate was found in the perfusate, indicating that little if any salicylate was present in the rectal membranes after intraveous infusion of salicylate. Although salicylate is readily absorbed from the rectum to the plasma, the reverse does not occur under these conditions. It also appears that salicylate does not promote rectal drug absorption except when it is present in the rectal tissue. This is supported by the observation (2) that the enhancement of theophylline absorption from the rectum by salicylate was eliminated by washing the rectum with a buffer solution after pretreatment with salicylate. This is in contrast to the effect of sodium lauryl sulfate which continued after washing the rectum following pretreatment with sodium lauryl sulfate.

It appears that salicylate interacts with some feature of the rectal membrane facilitating the transport of drug substances from the rectum

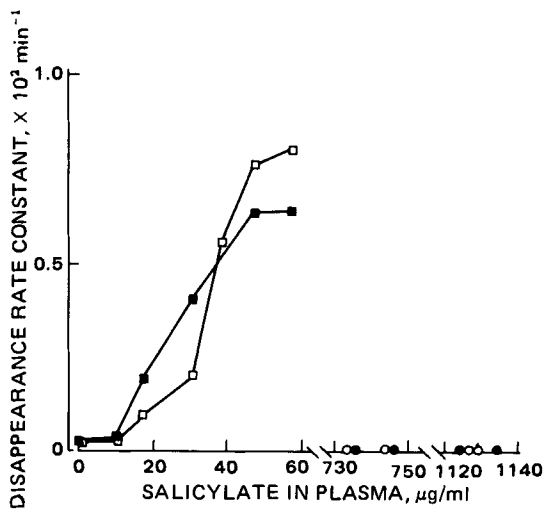


**Figure 10**—*Disappearance rate constant (× $10^2$ $min^{-1}$) of cefmetazole (■) and levodopa (□) from perfusate as a function of salicylate concentration in the plasma following rectal administration. High plasma concentrations of salicylate following intravenous salicylate administration did not result in significant disappearance of cefmetazole (●) or levodopa (○).*

to the general circulation. Studies are continuing on the mechanism of this action.

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# Analysis of Iodochlorhydroxyquin in Cream Formulations and Bulk Drugs by High-Performance Liquid Chromatography

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Received June 6, 1981, from the *Quality Control Division, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication October 23, 1981.

**Abstract** □ A high-performance liquid chromatographic method for the analysis of iodochlorhydroxyquin in creams and as bulk drugs has been developed. Iodochlorhydroxyquin was acetylated in the 8-position by reaction with acetic anhydride in pyridine. The resulting ester was mixed with the internal standard and chromatographed on a microparticulate silica column. Recovery was quantitative and the method was shown to be applicable to cream formulations from several manufacturers.

**Keyphrases** □ Iodochlorhydroxyquin—analysis in cream formulations and bulk drugs, high-performance liquid chromatography □ High-performance liquid chromatography—iodochlorhydroxyquin, analysis in cream formulations and bulk drugs □ Cream formulations—analysis of iodochlorhydroxyquin and bulk drugs by high-performance liquid chromatography

Iodochlorhydroxyquin (5-chloro-7-iodo-8-hydroxyquinoline) (I) has antifungal and antibacterial activities and is used in the treatment of inflamed skin conditions such as eczema, athlete's foot, and other fungal infections. Its use is generally limited to topical applications and is commercially available in lotion, cream, and ointment formulations. It is frequently formulated in combination with the corticosteroid, hydrocortisone. Monographs for



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