cedures (HPLC and GC) for determining dextromethorphan hydrobromide from other commercially available cough syrups. Chromatograms obtained for these products were representative of the assay procedure and gave resolution and/or separation similar to that of dextromethorphan hydrobromide in the products under investigation. Typical chromatograms are shown in Figs. 3 and 4 for HPLC and GC, respectively, and the results are compared in Table IV.

The results obtained by the GC procedure were higher for all the products analyzed and, hence, reflected the difficulty in extracting the free base with organic solvents (10). This difficulty was experienced with the standard and sample solutions as well. It is believed that the partitioning behavior (11) of the antitussive agent is the major cause for this problem rather than the complexity of the cough syrup formulations. The GC procedure involved a lengthy sample preparation and required more analysis time as compared with the HPLC procedure. Finally, the HPLC procedure gave results that were in better agreement with label claims for all the products evaluated and was a more precise assay procedure.

CONCLUSION

Although excellent resolution of the antitussive agent was obtained by both the HPLC and GC systems, further examination of the applicability of either method for routine analysis of dextromethorphan hydrobromide revealed that the HPLC procedure described is simple, rapid, and precise for the quantitation of the antitussive agent in several commercially available cough-cold syrups. The GC procedure does not seem to be the method of choice for quantitation of dextromethorphan hydrobromide in cough syrups due to the difficulty in extracting the free base and the time involved in sample preparation.

REFERENCES

(1) Alfred Burger, "Medicinal Chemistry," part II, 3rd ed., Wiley-Interscience, New York, N.Y. 1970.

(2) R. Grewe and A. Mondon, *Chem. Ber.*, **91**, 279 (1948).

(3) E. J. Kubiak and J. W. Munson, *J. Pharm. Sci.*, **69**, 1380 (1980).

(4) J. L. Fabregas and A. Margalet, *J. Pharm. Sci.*, **64**, 1005 (1975).

(5) E. R. Kaplan and A. A. Spark, *So. African J. Sci.*, **71**, 241 (1975).

(6) G. W. Halstead, *J. Pharm. Sci.*, **71**, 1108 (1982).

(7) G. W. Schieffer and D. E. Hughes, *J. Pharm. Sci.*, **72**, 55 (1983).

(8) W. O. McSharry and I. V. E. Savage, *J. Pharm. Sci.*, **69**, 212 (1980).

(9) M. K. Chao, I. J. Holcomb, and S. A. Fusari, *J. Pharm. Sci.*, **68**, 1463 (1979).

(10) A. Menyhareth, F. P. Mahn, and J. E. Heveran, *J. Pharm. Sci.*, **63**, 431 (1974).

(11) T. Higuchi *et al.*, *Anal. Chem.*, **39**, 974 (1967).

(12) P. Mukerjee, *Anal. Chem.*, **28**, 870 (1956).

Nasal Drug Delivery System of a Quaternary Ammonium Compound: Clofilium Tosylate

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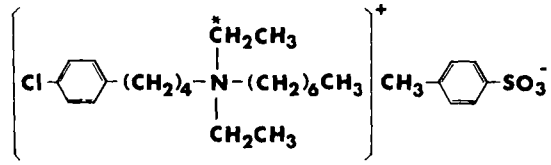
Received September 9, 1983, from the *Pharmaceutical Research Department and Pathology Division, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.* Accepted for publication October 12, 1983.

Abstract □ The blood levels of the [^{14}C]clofilium ion in rats after various routes of administration of clofilium tosylate were compared. The results indicate that the blood levels after nasal administration were not statistically different from levels after intravenous administration ($p > 0.05$). Administration by the oral route resulted in considerably lower blood levels. Nasal administration of clofilium tosylate appeared to be superior to oral administration. Histological examinations of nasal mucosa were conducted. At the lower concentration, mild necrosis was observed, and large areas of mucosa were unaffected. However, necrosis of large areas of mucosa occurred after exposure to the higher concentration. Levels of radioactivity in heart, liver, lung, and kidney tissue, as a function of time, were also studied. Unlike the blood levels after nasal administration, the levels of radioactivity were persistent in heart tissue. The data suggest that the [^{14}C]clofilium ion and/or metabolite concentrate in the heart and that blood levels of radioactivity may not be an accurate index of cardiac levels or biological response.

Keyphrases □ Clofilium tosylate—nasal drug delivery □ Drug delivery systems—clofilium tosylate, nasal administration

Clofilium tosylate, [4-(*p*-chlorophenyl)butyl]diethylheptylammonium tosylate, is a newly synthesized quaternary ammonium compound that has been shown to selectively increase refractoriness of cardiac tissue in dogs and humans (1-3). It is known that oral administration of quaternary ammonium compounds results in low and varied blood levels (4). Predictably, the oral absorption of clofilium tosylate in rats was poor (5). A total of 0.35% of the dose was excreted in the urine and 79% was excreted in the feces within 72 h after oral administration. All tissues had extremely low levels of radioactivity and low rates of absorption after oral administration of clofilium tosylate. The pharmacokinetics and bioavailabilities of quaternary ammonium compounds given by various routes have been studied previously (4-7). However, nasal administration of a quaternary ammonium compound has not yet been evaluated. It was the object of this study to report the results on the nasal absorption of clofilium tosylate in rats. The tissue disposition of radioactivity after nasal administration of [^{14}C]clofilium tosylate is also reported.



EXPERIMENTAL SECTION

Synthesis of [^{14}C]Clofilium Tosylate—[^{14}C]Clofilium tosylate was prepared in this laboratory by Dr. F. J. Marshall. *N*-[^{14}C]Acetyl-*N*-heptyl-(4-chlorophenyl)butylamine was prepared as follows: 104.4 mg (1.33 mmol) of [^{14}C]acetyl chloride was introduced by vacuum transfer into a flask containing 804.8 mg (2.86 mmol) of 4-(4-chlorophenyl)butylamine in 13 mL of dry toluene. The mixture was then stirred for 3 h and warmed gently for 1 h in a stoppered flask. The solution was washed with 10 mL of a 1 M HCl-water-saturated sodium chloride solution and then dried with magnesium sulfate and the organic phase was evaporated to dryness under reduced pressure. The resulting material was dissolved in chloroform and purified by preparative TLC (ethyl acetate, silica gel[1]). A total of 277 mg (64% yield) of the amide was obtained. This material was reduced to the tertiary amine as follows. With cooling in ice water, a solution of 277 mg (0.855 mmol) of the aforementioned

[1] 60-F254; E. Merck, Darmstadt, Federal Republic of Germany.

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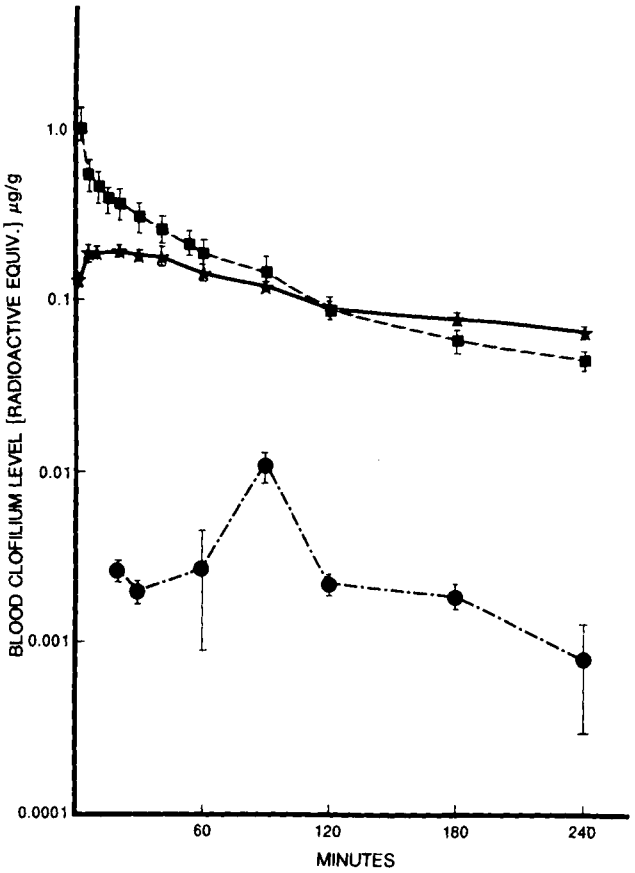

Figure 1—*[^{14}C]Clofilium ion in blood of male Sprague-Dawley rats after various routes of administration of a 1.2-mg/kg dose (n = 3). Key (1) intranasal administration; (2) intravenous administration; (3) oral administration.*

amide in 8 mL of tetrahydrofuran was added dropwise to 5.25 mL of a 1 M solution of diborane in tetrahydrofuran. The mixture was refluxed gently for about 20 h, cooled in ice water, and 7 mL of methanol was cautiously added. The mixture was stirred for a few minutes, and 11 mL of 1 M HCl and 3 mL of concentrated HCl were added. The mixture was heated for 3 h. After cooling in ice water, it was made strongly basic by the addition of 5 M NaOH. The product was extracted with ether, and the extract was washed with water-saturated sodium chloride solution and dried with magnesium sulfate. The ether was removed under reduced pressure. The tertiary amine was purified by preparative TLC as described above (ethyl acetate-triethylamine, 30:1) to give 206 mg (78% yield) of material which showed one spot on TLC. The quaternary tosylate was prepared as follows: 206 mg (0.665 mmol) of the ^{14}C-labeled tertiary amine was refluxed with 159.6 mg (0.797 mmol) of ethyl-*p*-toluenesulfonate in 3.2 mL of 1,2-dichloroethane for 96 h. The solvent was removed under reduced pressure, and the residue was recrystallized from acetone-ethyl acetate to give 226 mg of colorless crystals of [^{14}C][4-(*p*-chlorophenyl)butyl]diethylheptylammonium tosylate, mp 98–101°C. The product appeared to be 97.5% pure by TLC and autoradiography.

Formulation—[^{14}C]Clofilium tosylate was dissolved in NaCl for injection USP (8) and sonicated before administration to rats.

Animal Absorption Studies—Male Sprague-Dawley rats (average weight, 275 g) were anesthetized during the entire course of the intravenous and nasal absorption studies. For intravenous administration, the rats were anesthetized with pentobarbital by intraperitoneal injection, and the drug solution was administered by bolus injection into the femoral vein. For nasal administration, the surgical procedure conducted on the rats was that described previously (9, 10) with some modifications. The drug solution was delivered to the nasal cavity through the esophagus cannulation tubing by means of a syringe. For oral administration, the rats were not anesthetized, and the drug solution was administered by gavage. Blood samples were taken from the tail vein periodically and acid-digested in 0.2 mL of 70% $HClO_4$ and 0.4 mL of 30% H_2O_2 at 70°C for 1 h in scintillation vials. The samples were cooled and counted in scintillation fluid[2].

[2] Ready-Solv MP; Beckman.

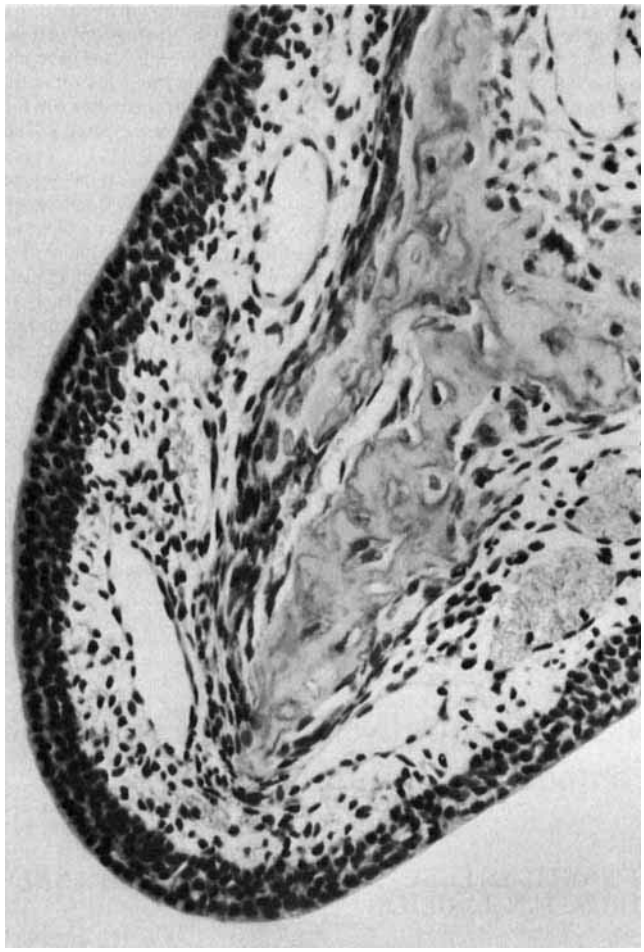

Figure 2—*Nasal mucosa of necropsy A; control rat.*

Histological Examination of Nasal Mucosa in Rats—After the absorption phase of the study, the rats were killed, decapitated, and the nasal passages were flushed with 10% buffered formalin. After fixation by immersion, the heads were decalcified, and four cross sections of the nasal cavity of each rat were stained and examined (11).

Tissue Distribution of the [^{14}C]Clofilium Ion after Nasal Administration of [^{14}C]Clofilium Tosylate—At various times after nasal administration of the drug, tissues were dissected, rinsed, blotted, and weighed. Samples (100–260 mg) were digested in 0.2 mL of 70% $HClO_4$ and 0.4 mL of 30% H_2O_2 at 70°C for 1 h in scintillation vials. The samples were cooled and counted in scintillation fluid[2].

RESULTS AND DISCUSSION

The disappearance of radioactivity in the blood of rats administered [^{14}C]clofilium tosylate by the three administration routes is shown in Fig. 1. The variations in blood levels as well as the low absorption rate following administration of the oral dose are clearly demonstrated. Nasal administration of the drug resulted in much higher levels of radioactivity than levels after oral administration. The blood radioactivity levels of the clofilium ion after oral administration of the tosylate were ~1% of the levels obtained from the dose, whereas the radioactivity levels after nasal administration were 70% of those obtained from the dose (Table I). Furthermore, the difference in levels following intravenous and nasal administrations were statistically insignificant ($p > 0.05$).

The data on the effect of the clofilium ion concentration indicate that the area under the curve of the blood radioactivity level increased with dose in a nonlinear manner when administered nasally (Table II). However, a linear dose-response relationship appeared in the lower ranges. The nonlinear portion of the curve and good absorption rate at the concentration of 1.2 mg/kg were possibly attributable to damage to the nasal mucosa with exposure of the submucosal vasculature to the quaternary ammonium compound. Inspection of the blood kinetics of the radioactivity levels (Table III) suggested that, even at this lower dose, the drug was slowly absorbed and/or diffused through epithelial cells into the systemic circulation. At 0.3–0.6 mg/kg, the absorption

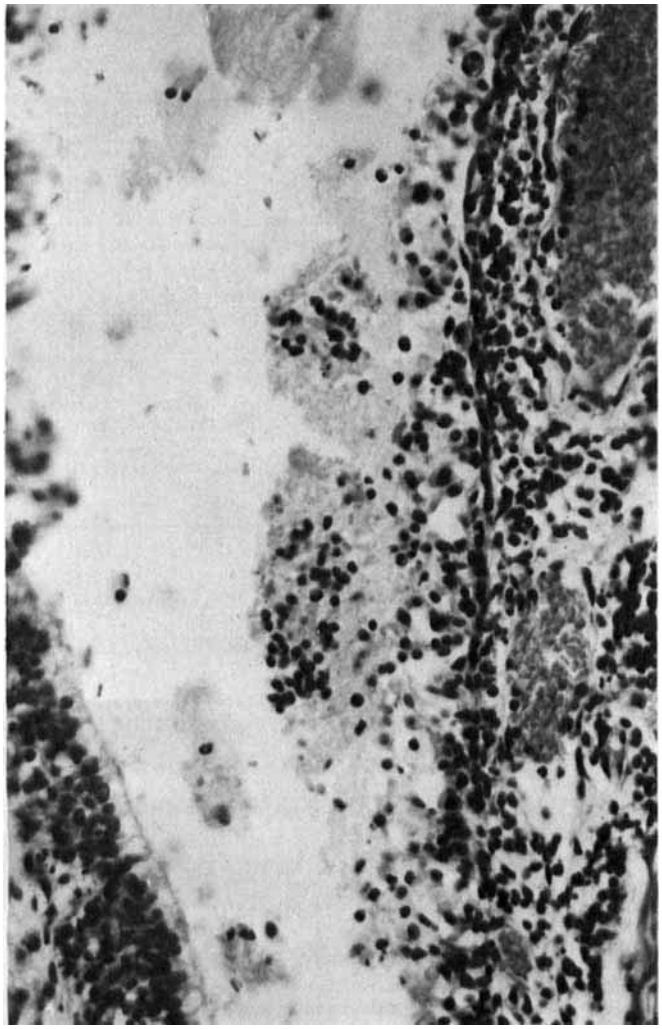

Figure 3—*Nasal mucosa of necropsy B; the rat was given a 1.2-mg/kg dose.*

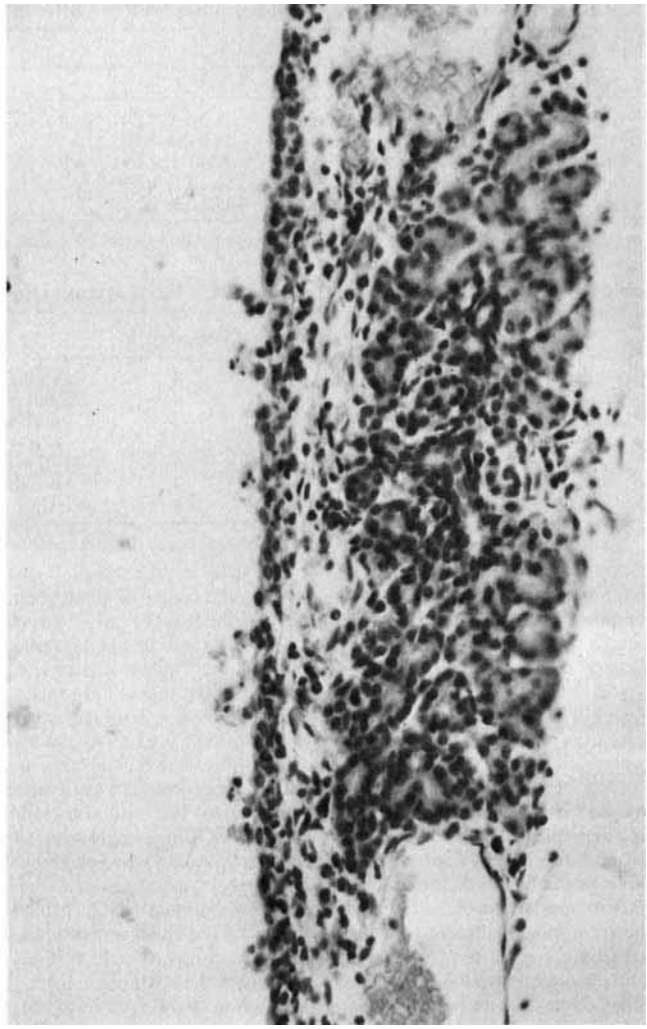

Figure 4—*Nasal mucosa of necropsy C; the rat was given a 0.6-mg/kg dose.*

under the AUC per unit dose remained constant (Table II). Thus, the amount of drug absorbed was directly proportional to the concentration in accordance with the law of diffusion. The observations are in agreement with those reported previously; that is, quaternary ammonium compounds are absorbed by a diffusion mechanism (6). Still, by administering a 0.3-mg/kg dose nasally, the nasal absorption was ~21-fold greater than the oral absorption. In these studies, it was indicated that clofilium tosylate was rapidly absorbed from the nasal mucosa. The peak blood level was attained ≤10 min after nasal instillation. A nasal drug delivery system for quaternary ammonium compounds appears to be feasible for this antiarrhythmic agent or for other applications.

Quaternary ammonium salts are often used as surface-active agents when dissolved in water for antimicrobial and/or antiseptic purposes (12, 13). The possibility that nasal absorption of clofilium tosylate could be attributable

Table I—Comparison of [^{14}C]Clofilium Ion Blood Levels [a] in Rats [b] after Various Routes of Administration

Route of Administration	Dose, mg/kg[c]	AUC, μg·min/g[d]	Absorption, %[e]	p[f]
Intravenous	1.2	39.5 ± 4.98	—	—
Oral	1.2	0.53[g] ± 0.097	1.3	0.001
Nasal	1.2	27.5[h] ± 1.30	69.6	0.08

[a] Expressed as microgram equivalents of carbon-14 per gram of blood. [b] Harlan Sprague-Dawley male rats, $n = 3$. [c] Formulated in solution form. [d] Mean ± *SEM* of three rats. [e] Percentage of intravenous dose. [f] Probability value (Student's t test), compared with intravenous administration route data. [g] $p < 0.05$, compared with nasal administration route data. [h] $p > 0.05$, compared with intravenous administration route data.

Table II—Comparison of [^{14}C]Clofilium Ion Blood Levels [a] in Rats—Effect of Dose

Route of Administration	Dose, mg/kg[b]	AUC, μg·min/g[c]	Mean Specific AUC[d]	Absorption, %
Intravenous	1.2	39.5 ± 4.98	32.9	—
Nasal	1.2	27.5 ± 1.30	22.9	69.6
Nasal	0.6	5.70 ± 1.06	9.50	28.9
Nasal	0.3	2.81[e] ± 0.28	9.37	28.5

[a] Expressed as microgram equivalents of carbon-14 per gram of blood. [b] Formulated in solution form. [c] Mean ± *SEM* of three rats. [d] Mean specific AUC: (μg·min/g)/(mg/kg) = mean AUC/administered dose. [e] $p > 0.05$, compared with an AUC of 0.6 mg/kg.

Table III—Tissue Distribution [a] of [^{14}C]Clofilium Ion [b] in Rats [c] after Intranasal Administration of a 0.3-mg/kg Dose of Clofilium Tosylate

Tissue	Time after Dose, h				
	0.5	1	2	4	6
Blood	12.06 ± 1.80	9.30 ± 0.34	14.88 ± 1.44	11.80 ± 1.20	14.40 ± 3.80
Heart	82.12 ± 12.54	88.09 ± 4.10	1785.16 ± 23.10	227.80 ± 22.50	263.70 ± 27.00
Lung	58.95 ± 8.42	49.47 ± 4.69	83.88 ± 8.90	97.06 ± 15.20	112.10 ± 15.00
Liver	221.31 ± 29.19	218.24 ± 12.20	328.60 ± 36.30	482.90 ± 39.80	271.90 ± 36.00
Kidney	475.13 ± 87.60	609.42 ± 20.59	944.40 ± 85.50	1116.00 ± 106.80	813.00 ± 110.00

[a] Each value represents the mean ± *SEM* of three animals. [b] Expressed as nanogram equivalents of carbon-14 per gram of tissue. [c] Harlan Sprague-Dawley male rats; $n = 3$.

Table IV—Summary of Histological Examination of Nasal Mucosa after Intranasal Administration of Clofilium Tosylate to Rats

Necropsy	Dose, mg/kg	Exposure, h	Microscopic Findings[a]
A	Control	0	No significant lesions.
B	1.2	6	About one-half of the mucosa was necrotic or missing. Occasional microabscesses were present in the mucosa. The lumens of affected turbinates contained debris formed of neutrophils and sloughed mucosa.
C	0.6	6	Mild necrosis was characterized by occasional eosinophilic, round, mucosal cells with pycnotic nuclei. Minimal amount of luminal debris.
D	0.3	6	Similar to that for the 0.6-mg/kg dose.

[a] Summarized from the observation from four cross sections of the nasal cavity of each rat and from a total of three rats in each study.

to damage to the mucosa was realized during the course of these studies. Therefore, histopathological studies were conducted. To avoid physical damage to any part of the nasal mucosa, the drug solution was delivered to the nasal cavity through the esophagus cannulation tubing with a syringe. Typical lesions of the exposed specimens of rat nasal mucosa are reported (Figs. 2-4). In general, large portions of mucosa that were in contact with the highest concentration of clofilium tosylate became necrotic. At the lower concentrations, only occasional epithelial cells were necrotic, and large areas of mucosa were unaffected. No histological differences between nasal mucosa exposed to the 0.3- or 0.6-mg/kg dose were detected. The results observed in the nasal mucosa of rats after administration of clofilium tosylate are summarized in Table IV. Control groups were also exposed to the vehicle.

The time course of the clofilium ion radioactivity in various tissues following nasal administration of clofilium tosylate in rats is summarized in Table III. The absorption kinetics of the radioactivity from the blood contrasted with that from some tissues. One hour after nasal administration, high levels of radioactivity relative to the blood were observed in the heart, lung, liver, and kidney tissues. There was a gradual accumulation of the drug in the heart, with a peak occurring between 4 and 6 h based on the experimental design. Radioactivity levels in the heart appeared to be very persistent. By 6 h after nasal administration, the total radioactivity in the heart was about 18-fold greater than that in the blood. The results are consistent with the previously reported gradual uptake by the myocardium after intravenous administration of the drug in rats and dogs (14). Distribution in blood and the relationship between tissue and blood concentrations appear to be similar, regardless of whether administration is intravenous or nasal. Other quaternary ammonium antiarrhythmic agents, such as bretylium and 1-(dimethylisopropylamino)-3-(1-naphthyloxy)-2-propanol methochloride (UM-272), are also known to have a high affinity for myocardial tissue relative to plasma (15, 16). The biological response of clofilium tosylate correlates with the levels of radioactivity in the heart (14). These data re-emphasize the fact that for the clofilium ion, and possibly other quaternary ammonium antiarrhythmic agents, blood levels are an insufficient indicator for estimating cardiac levels and thus pharmacological effect.

REFERENCES

(1) M. I. Steinberg and B. B. Molly, *Life Sci.*, **25**, 1397 (1979).

(2) M. I. Steinberg, M. E. Sullivan, S. A. Wiest, F. W. Rockhold, and B. B. Molly, *J. Cardiovasc. Pharmacol.*, **3**, 881 (1981).

(3) E. Platia and P. R. Reid, *Circulation*, **62**, 153 (1980).

(4) E. R. Garrett, J. R. Green, and M. Bialer, *Biopharm. Drug Dispos.*, **3**, 129 (1982).

(5) M. I. Steinberg, in "New Drug Annual," Raven Press, New York, N.Y., 1984, p. 103.

(6) J. A. Hemberger and L. S. Schanker, *Drug Metab. Dispos.*, **II**, 73 (1983).

(7) B. L. Kamath, H. F. Stampfli, C. M. Lai, and A. Yacobi, *J. Pharm. Sci.*, **70**, 667 (1981).

(8) "U.S. Pharmacopeia XX," U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 727.

(9) A. Hussain, S. Hirai, and R. Bawarshi, *J. Pharm. Sci.*, **69**, 1411 (1980).

(10) S. Hirai, T. Yashiki, and T. Matsuzawa, presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, April 1978.

(11) J. T. Young, *Fund. Appl. Toxicol.*, **1**, 309 (1981).

(12) R. M. E. Richards and R. H. Cavill, *J. Pharm. Sci.*, **65**, 76 (1976).

(13) J. P. Remington, in "Remington's Pharmaceutical Sciences," 14th ed., Mack Publishing Co., Easton, Pa., 1970, p. 315.

(14) T. D. Lindstrom, P. J. Murphy, J. K. Smallwood, S. A. Wiest, and M. I. Steinberg, *J. Pharmacol. Exp. Ther.*, **221**, 584 (1982).

(15) D. H. Namm, C. M. Wang, S. El-Sayad, F. C. Capp, and R. A. Maxwell, *J. Pharmacol. Exp. Ther.*, **193**, 194 (1975).

(16) E. Patterson, P. Stetson, and B. R. Lucchesi, *J. Pharmacol. Exp. Ther.*, **214**, 449 (1980).

ACKNOWLEDGMENTS

This study was presented to the Basic Pharmaceutics Section, American Pharmaceutical Association Academy of Pharmaceutical Sciences 35th National Meeting, Miami Beach, Fla., November 1983.

The authors thank Dr. T. D. Linstrom for helpful discussions and Mrs. C. Lammers for assistance in preparing the manuscript.