# Comparative Bioavailability of Four Commercial Quinidine Sulfate Tablets

JEFFREY D. STRUM, JOHN L. COLAIZZI ×, JAMES M. JAFFE, PERRY C. MARTINEAU *, and ROLLAND I. POUST

**Abstract** □ A comparative bioavailability study was performed using four commercially available, chemically equivalent brands of quinidine sulfate tablets. Two 200-mg tablets were administered to 11 different subjects following a completely randomized crossover design. Serum levels, urinary excretion data, and derived pharmacokinetic parameters were compared statistically. There were no statistical differences in the extent of quinidine absorption from the four brands of tablets as evidenced by the cumulative urinary excretion values and the areas under the serum level–time curves. Significant differences in the mean serum levels at 0.5 and 1 hr and differences in the peak times and absorption rate constants indicate that there was a difference in the absorption rate between Treatments A and D and C and D. A significant difference in the peak times also was noted for Treatments B and C. When mean disintegration times for the four tablet formulations were compared with their values for $k_a$, $t_{max}$, and mean serum levels at 0.5 and 1 hr, rank-order correlations were observed. A considerable degree of variability in quinidine elimination was noted, with half-life values ranging from 2.71 to 8.12 hr (mean half-life of 5.36 hr).

**Keyphrases** □ Quinidine sulfate—bioavailability of four commercial tablets compared, humans □ Bioavailability—quinidine sulfate, four commercial tablets compared, humans □ Cardiac depressants—quinidine sulfate, bioavailability of four commercial tablets compared, humans

Emphasis has been placed recently on the importance of establishing the biological equivalence of quinidine sulfate tablets. The APhA Academy of Pharmaceutical Sciences classified quinidine sulfate as one of the "commonly prescribed multiple source drugs whose solid dosage forms exhibit the most serious bioavailability and/or quality assurance problems" (1). However, since no published articles report the comparative bioavailability of chemically equivalent quinidine sulfate tablets, the classification of the Academy of Pharmaceutical Sciences must be taken to mean that there is a potential for bioavailability differences with this drug dosage form.

The Food and Drug Administration (FDA) also indicated that quinidine sulfate is one drug for which a bioequivalence requirement is currently anticipated and specified that *in vivo* testing would be necessary to compare different commercial products (2). A list of approved manufacturers and distributors of bioequivalent problem drugs recently published by the FDA included four manufacturers[1] and one distributor[2] of quinidine sulfate tablets (3).

## BACKGROUND

Quinidine is a frequently prescribed antiarrhythmic drug. In 1975, it ranked 14th among the most frequently prescribed generic drugs in the United States (4). Currently, at least 60 different generic brands of 200-mg quinidine sulfate tablets are on the market (5). Studies established the blood level profiles of several salt and sustained-release dosage forms of quinidine to define an optimal dosage regimen (6–11). Other reports compared the blood levels produced by administering quinidine by different routes (12). The bioavailability of the drug is variable, especially when taken with food, since it is known to interact with bile anions to form an insoluble complex (13).

A specific range of serum quinidine levels (3–6 μg/ml) must be maintained to achieve the desired pharmacological effect (14). This therapeutic range varies, however, depending upon the assay methodology employed and may be as low as 2–4 μg/ml (11). Several studies indicated that there is a definite correlation between serum levels and clinical effect (15–17). However, a recent report (18) stressed cautious interpretation of serum level when predicting therapeutic or toxic response since individual patient sensitivity to the drug may be more important. Toxic reactions may occur at any serum level due to allergy or idiosyncrasy but are more likely to appear at levels above 8 μg/ml (19, 20).

Studies employing intravenous infusions of quinidine gluconate defined the kinetic disposition of the drug and showed that there is considerable interpatient variability in quinidine elimination (21). A recent study[3] employing capsule, tablet, and solution forms of quinidine sulfate and an intramuscular injection of the gluconate confirmed that there is a high degree of intersubject and intrasubject variability in the biological half-life of the drug as well as a difference in the bioavailability of the tablet and intramuscular dosage forms.

Based on the low therapeutic index of this drug, the critical purpose for which it is indicated, its reported potential for bioequivalency problems, its wide generic availability, and the lack of published comparative bioequivalency data, the objective of this study was to compare the bioavailability of four commonly available, chemically equivalent brands of quinidine sulfate tablets.

## EXPERIMENTAL

***In Vivo* Study Protocol**—Eleven healthy, normal adult male volunteers, 20–37 years of age, were the subjects. The treatments consisted of four different brands of quinidine sulfate tablets acquired by the normal channels of drug distribution. A dose of 400 mg of quinidine sulfate (as two 200-mg tablets) was administered to each subject with approximately 180 ml of water. The subjects received each of the four treatments, A[4], B[5], C[6], and D[7], in a completely randomized crossover design. A 2-week period was employed between each dosage interval to allow for complete drug elimination. All subjects fasted for 8 hr before and 3 hr following drug administration.

Blood samples (6 ml) were drawn using evacuated glass containers[8] just prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 32 hr following drug administration. The samples were immediately centrifuged[9] at 3000 rpm, and the serum was separated and analyzed for quinidine. Complete urine voids were collected at intervals of 2, 4, 6, 8, 12, 16, 24, 28, 32, 40, 48, 58, and 72 hr following drug administration and analyzed for quinidine.

***In Vitro* Assessment of Dosage Forms**—Weight variation tests were performed on 20 tablets of each of the four brands following the method outlined in USP XIX (22) for determining permissible limits of weight variation.

Disintegration times were determined using an apparatus conforming

[1] Merrell-National, Parke-Davis, Phillips Roxane, and Rexall.
[2] Alliance.

[3] W. D. Mason, J. O. Covinsky, J. C. Valentine, K. C. Kelly, G. H. Weddle, and B. C. Martz, School of Medicine, University of Missouri, Kansas City, Mo., personal communication.
[4] Quinidine sulfate tablets USP, Lot 76F83A, Eli Lilly & Co., Indianapolis, Ind.
[5] Quinidine sulfate tablets USP, Lot 7088A, Phillips Roxane Laboratories, Columbus, Ohio.
[6] Quinora, Lot 72755, Lakeside Laboratories, Milwaukee, Wis.
[7] Quinidine sulfate tablets USP, Lot 15840, Stanlabs, Portland, Ore.
[8] Vacutainers, silicone-coated interior, Becton-Dickinson and Co., Rutherford, N.J.
[9] Clinical centrifuge model CL, International Equipment Co., Needham Heights, Mass.

Table I—Parameters Derived from Serum Levels and Urinary Excretion of Unchanged Drug

| Parameter | Treatment[a] A | B | C | D | Statistical Method | Statistical Evaluation |
|---|---|---|---|---|---|---|
| Mean value of peaks of individual serum concentration–time curves, μg/ml | 1.42 | 1.45 | 1.43 | 1.33 | ANOVA | NS[b] |
| Mean time of peak value of individual serum concentration–time curves, hr | 1.84 | 2.27 | 1.63 | 2.54 | Wilcoxon's matched-pairs signed ranks test | $p < 0.05$ |
| Mean corrected area under individual serum concentration–time curves, (μg/ml) × hr | 14.41 | 15.80 | 15.11 | 14.63 | ANOVA | NS |
| Mean $k_a$ value of individual serum concentration–time curves, $hr^{-1}$ | 2.88 | 2.08 | 2.91 | 1.12 | Wilcoxon's matched-pairs signed ranks test | $p < 0.05$ |
| Mean $K_E$ value of individual serum concentration–time curves, $hr^{-1}$ | 0.142 | 0.130 | 0.125 | 0.138 | Wilcoxon's matched-pairs signed ranks test | NS |

[a] See text for explanation of treatment conditions. [b] NS = not statistically significant.

to USP specifications[10] and employing six tablets of each of the four products. Tablets were tested according to the official specifications with purified water maintained at 37 ± 2° as the immersion fluid.

Although a content uniformity test is not specified for quinidine sulfate tablets, the relative potency of the tablets was determined by performing individual assays on each of 10 randomly chosen tablets of the four different brands. An additional 10 tablets of each brand were chosen and collectively assayed for mean quinidine content. The assay was conducted by following the nonaqueous titration procedure outlined in USP XIX (23) for quinidine sulfate tablets.

**Fluorometric Determination of Quinidine in Biological Fluids**—The analytical method used for the determination of quinidine in serum and urine was a modified form of the fluorometric assay developed by Cramer and Isaksson (24). This method utilizes a double-extraction procedure, which limits the extraction of water-soluble metabolites of quinidine and dihydroquinidine. Fluorescence was determined on a spectrofluorometer[11] at 450-nm emission and 340-nm excitation. Stock solutions for the calibration plots were prepared such that results are reported in terms of quinidine base.

**Statistical Evaluation of Results**—The following parameters were analyzed for variance for a crossover design using a computerized statistical program (BMD08V) (25): (*a*) serum concentrations at each time period, (*b*) peak serum concentrations, (*c*) corrected area under the serum concentration–time curve, and (*d*) total urinary excretion of the unchanged drug. Any variance established among treatments was further analyzed for differences between all possible treatment pairs using Tukey's allowable difference test (26).

Wagner (27) and Westlake (28) pointed out that univariate parameters, such as absorption rate constants and elimination rate constants estimated from serum level–time data, should not be analyzed by methods assuming uniform error variance. Therefore, values for the absorption rate constant, $k_a$, elimination rate constant, $K_E$, and calculated peak time, $t_{max}$, were analyzed nonparametrically using Wilcoxon's matched-pairs signed ranks test (29).

## RESULTS

Based on the shapes of semilogarithmic plots of individual serum concentration–time data for the 11 subjects, the data were fitted to the classical one-compartment model, both with and without a lag time (30, 31), using a nonlinear least-squares regression computer program (32) on a digital computer[12]. Comparison of the values of the individual sums of squares of the deviations between experimental data and calculated values showed the one-compartment model with a lag time to have the better fit. The individual areas under the serum concentration–time curves (*AUC*) were calculated using the trapezoidal rule, including a correction for the *AUC* beyond the last data point. The peak time is the sum of the calculated peak time and the calculated lag time, $t_{lag}$. The calculated peak time was determined using (33):

$$t_{max} = \frac{2.303}{k_a - K_E} \log \frac{k_a}{K_E} \quad \text{(Eq. 1)}$$

The lag time was calculated from (34):

$$t_{lag} = \frac{2.303(\log A_2 - \log A_1)}{k_a - K_E} \quad \text{(Eq. 2)}$$

where $A_2$ and $A_1$ represent the coefficients for the exponential terms describing absorption and elimination, respectively.

Table I summarizes the pharmacokinetic data obtained from the individual serum and urinary excretion data. An analysis of variance and Wilcoxon's matched-pairs signed ranks test were performed on the appropriate parameters. A statistical difference was evidenced for the time of the peak concentration of the individual serum concentration–time curves between Treatments A and D, B and C, and C and D at the 95% level of confidence. Statistical differences ($p < 0.05$) were also found between Treatments A and D and C and D for the $k_a$ values derived from the individual serum concentration–time curves.

Figure 1 illustrates the mean cumulative urinary excretion of the unchanged drug for the four brands of tablets. Statistical analyses (ANOVA)

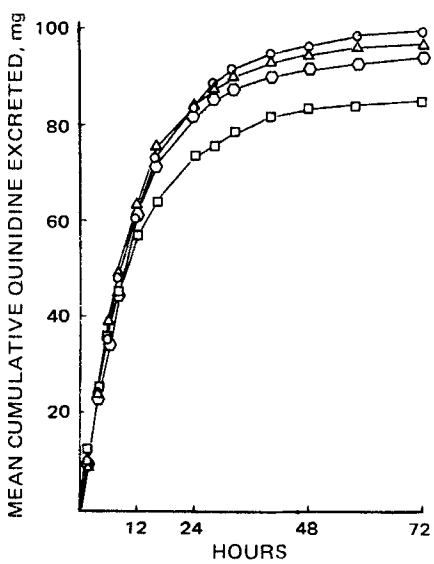


**Figure 1**—*Mean cumulative milligrams of unchanged quinidine excreted in urine (0–72 hr). Key: ○, Treatment A; △, Treatment B; □, Treatment C; and ○, Treatment D.*

[10] Van Kel Industries, Livingston, N.J.
[11] Turner model 430, G. K. Turner Associates, Palo Alto, Calif.
[12] PDP-10, Digital Equipment Corp., Maynard, Mass.

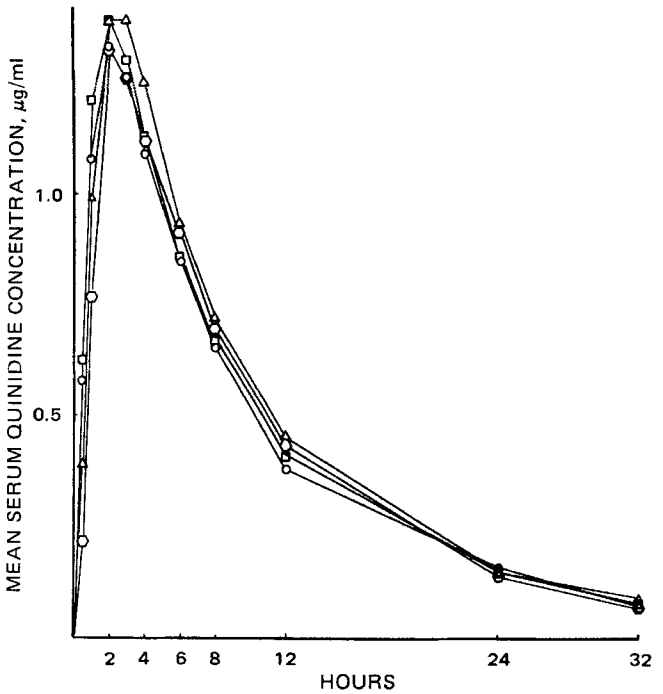


**Figure 2**—*Mean serum concentrations for 11 subjects for four different brands of quinidine sulfate tablets (0–32 hr). Key: ○, Treatment A; △, Treatment B; □, Treatment C; and ◇, Treatment D.*

revealed no differences between the four tablet brands based on the urinary excretion data. Figure 2 represents the mean serum level values for each treatment at each of the 10 sampling times following drug administration. An analysis of variance revealed a statistical difference ($p < 0.05$) among the treatment values for the mean serum values obtained at 0.5 and 1.0 hr following drug administration.

Differences among treatments were analyzed to determine the difference between treatment pairs using Tukey's test. A statistical difference was evidenced between Treatments A and D and C and D at 0.5 hr. There was also a statistical difference between Treatments C and D at 1.0 hr. The mean elimination half-life was calculated using (35):

$$t_{1/2} = \frac{0.693}{K_E} \qquad \text{(Eq. 3)}$$

and the individual $K_E$ values were obtained from the computer program. The mean $t_{1/2}$ for all subjects and all treatments was 5.36 ± 1.07 hr, with all values falling into a range of 2.71–8.12 hr.

All four brands passed the weight variation and disintegration tests for quinidine sulfate tablets as specified in USP XIX. Mean disintegration times were 4.08 ± 0.20 min for Treatment A, 7.16 ± 1.94 min for Treatment B, 1.00 ± 0.0 min for Treatment C, and 17.83 ± 2.22 min for Treatment D. The relative potency of the individual tablets was also confirmed, with all individual tablet assays for all four brands resulting in 86.7–104.9% of the average of the monograph potency definition for the drug (36). The data confirmed the assumption that the various brands were chemical equivalents.

## DISCUSSION

The bioavailability of a drug from tablet dosage forms depends on both the rate and extent of drug absorption into the general circulation. These factors can be evaluated by examining the pharmacokinetic parameters derived from blood level–time profiles and urinary excretion patterns for the unchanged drug. Bioequivalence is assured when the serum level–time curves for different brands of tablets are superimposable. Bioequivalence can also be established by comparing the peak serum concentrations of the drug, the times of the peak concentration, and the extent of absorption as reflected by the areas under the serum level–time curves.

Comparison of the area under the curve values revealed no significant differences in the extent of absorption of quinidine sulfate among the four different brands of tablets tested. The amount of drug excreted unchanged in the urine was also employed as an indicator of drug absorption. The urinary excretion of quinidine sulfate is pH dependent (37). Although no attempts were made to control urine pH in this study, comparison of the amounts excreted unchanged in the urine for 72 hr following administration showed no significant differences among the four brands tested. No apparent differences in urine pH values for the different treatments were noted.

The peak concentration and time of the peak concentration serve as indicators of the drug absorption rate. The peak concentration is generally achieved 2–3 hr following oral administration of a single dose of quinidine sulfate (38). Analyses of the data in Table I revealed a significant difference in the times of peak concentration between Treatments A and D, C and D, and B and C. A statistical difference also was evidenced for the mean serum levels between Treatments A and D and C and D at 0.5 hr and between Treatments C and D at 1.0 hr. Examination of the $k_a$ values also revealed significant differences between Treatments A and D and C and D. The treatment pairs that exhibited significant differences in mean serum levels (A and D and C and D) and $k_a$ values (A and D and C and D) also showed a statistical difference in the peak times (A and D and C and D), indicating that there was a difference in the absorption rate between these two formulation pairs. Treatment pair B and C also demonstrated a statistical difference in the peak time but not in serum levels or $k_a$ values.

When mean disintegration times for the tablet brands were compared with the values for $k_a$, $t_{max}$, and mean serum levels at 0.5 and 1.0 hr, rank-order correlations were observed. Treatment C, with the shortest mean disintegration time, had the largest $k_a$ value, highest serum levels at the first two sampling times, and the shortest $t_{max}$. Treatments A, B, and D followed in numerical ranking of mean disintegration times. There is currently no dissolution requirement in the compendial monograph of quinidine sulfate tablets, but dissolution studies are now underway in these laboratories to determine if a correlation between dissolution rates and *in vivo* parameters exist.

Although there was no significant difference among treatments for the mean biological half-life of the drug, there was considerable intersubject variability, in agreement with previously reported variability in the biological elimination of quinidine (39). The mean half-life for all subjects using all formulations was 5.36 ± 1.07 hr with a range of 2.71–8.12 hr. This range is less than that reported by Mason *et al.*[3] of 1.16–15.75 hr but corresponds more closely to the results of Ueda *et al.* (21), who reported a half-life of 6.33 hr with a range of 3.61–9.00 hr.

## REFERENCES

(1) *J. Am. Pharm. Assoc.*, **NS14,** 557 (1974).
(2) *Fed. Reg.*, **40,** 26168 (1975).
(3) *FDC Rep.*, **38,** B16 (1976).
(4) *Pharm. Times*, **42,** 37 (1976).
(5) "Drug Topics Red Book," Medical Economics Co., Oradell, N.J., 1976, pp. 356–357.
(6) W. M. Goldberg and S. G. Charkrabarti, *Can. Med. Assoc. J.*, **91,** 991 (1964).
(7) E. M. Ditlefson and H. E. Loken, *Acta Med. Scand.*, **179,** 333 (1966).
(8) G. Cramer, E. Varnauskas, and L. Werko, *ibid.*, **173,** 511 (1963).
(9) S. Bellet, D. Finkelstein, and H. Gilmore, *Arch. Intern. Med.*, **100,** 750 (1957).
(10) J. J. Sampson, H. Foreman, and B. Solomon, *Circulation*, **5,** 534 (1952).
(11) R. Henning and G. Nyberg, *Eur. J. Clin. Pharm.*, **6,** 239 (1973).
(12) R. W. Kalmansohn and J. J. Sampson, *Circulation*, **1,** 569 (1950).
(13) S. Riegelman, *Pharmacology*, **8,** 127 (1972).
(14) "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1971, p. 17.
(15) A. J. Lisenthal, S. Ulich, and L. A. Patterson, *J. Clin. Invest.*, **26,** 1188 (1947).
(16) A. B. Houston and W. F. Perry, *Can. Med. Assoc. J.*, **63,** 59 (1950).
(17) A. F. Delevett and C. A. Poindexter, *Am. Heart J.*, **32,** 697 (1946).
(18) J. P. Van Dueme and M. G. Bogaert, *Circulation*, **50,** 1284 (1974).
(19) H. L. Conn and R. J. Luchi, *Am. J. Med.*, **37,** 685 (1964).
(20) A. F. Lyon and A. C. DeGraff, *Am. Heart J.*, **70,** 140 (1965).
(21) C. T. Ueda, D. S. Hirschfeld, M. M. Scheinman, M. Rowland, B.

J. Williamson, and B. S. Dzindzio, *Clin. Pharmacol. Ther.*, **19**, 30 (1976).
(22) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1974, pp. 670, 671.
(23) *Ibid.*, p. 435.
(24) G. Cramer and B. Isaksson, *Scand. J. Clin. Lab. Invest.*, **15**, 553 (1963).
(25) P. Sampson, in "BMD, Biomedical Computer Programs," W. J. Dixon, Ed., University of California Press, Berkeley, Calif., 1974, pp. 693–704.
(26) D. U. Huntsberger and P. E. Leaverston, "Statistical Inference in the Biomedical Sciences," Allyn and Bacon, Boston, Mass., 1970, p. 213.
(27) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1975, p. 351.
(28) W. J. Westlake, *J. Pharm. Sci.*, **62**, 1585 (1973).
(29) N. M. Downie and R. W. Heath, "Basic Statistical Methods," 2nd ed., Harper and Row, New York, N.Y., 1965, pp. 237–239.
(30) G. A. Portmann, in "Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, pp. 4–6.
(31) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1975, p. 81.
(32) NLIN, Share Library No. 360 D-13.6.007, Research Triangle Park, NC 27709.
(33) G. A. Portman, in "Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1976, p. 17.
(34) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton Ill., 1975, pp. 81, 82.
(35) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1971, p. 252.
(36) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1974, p. 648.
(37) R. E. Gerhardt, R. F. Knouss, P. T. Thyum, R. J. Luchi, and J. J. Morris, *Ann. Intern. Med.*, **5**, 927 (1969).
(38) A. F. Lyon and A. C. DeGraff, *Am. Heart J.*, **69**, 834 (1965).
(39) K. M. Kessler, D. T. Lowenthal, H. Warner, T. Gibson, W. Briggs, and Reidenberg, *N. Engl. J. Med.*, **290**, 706 (1974).

**ACKNOWLEDGMENTS AND ADDRESSES**

Received March 26, 1976, from the *Department of Pharmaceutics, University of Pittsburgh, Pittsburgh, PA 15261.*

Accepted for publication June 11, 1976.

Abstracted from a thesis submitted by Jeffrey D. Strum to the University of Pittsburgh in partial fulfillment of the Master of Science degree requirements.

* Medical Laboratories, McKeesport Hospital, McKeesport, Pa.

[x] To whom inquiries should be directed.

# Mechanism of Histamine Binding II: Effect of Alkali Metal and Alkaline Earth Cations on Histamine Binding to Peptide H

**PAUL A. KRAMER, JOHN R. HAZLETT, and DANE O. KILDSIG [x]**

**Abstract** □ The association constants of histamine with $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, and $Ba^{2+}$ were determined in buffer solutions at constant pH using an ion selective electrode. These cations enabled histamine to bind to peptide H. A minimum cation binding concentration was required for histamine binding. A linear relationship existed between the minimum cation binding concentration and the log of the equilibrium constant for the histamine–cation complexes, indicating that the specificity of the alkaline earth cations in promoting histamine binding was due to the difference in their ability to complex with histamine. The monovalent cations, $Na^+$, $K^+$, and $Cs^+$, inhibited histamine binding to peptide H, with the extent of inhibition dependent on cation concentration. An ion-exchange mechanism or a conformation change in the peptide may account for the inhibition.

**Keyphrases** □ Histamine—binding to peptide H, effect of alkali metal and alkaline earth cations, association constants determined □ Peptide H—binding to histamine, effect of alkali metal and alkaline earth cations, association constants determined □ Alkali metal and alkaline earth cations—effect on binding of histamine to peptide H, association constants determined □ Binding—histamine to peptide H, effect of alkali metal and alkaline earth cations, association constants determined □ Association constants—determined for binding of histamine to peptide H, effect of alkali metal and alkaline earth cations □ Metals—alkali metal and alkaline earth cations, effect on binding of histamine to peptide H

Previous reports indicated that $Ca^{2+}$ is involved in the binding of histamine to serum protein (1, 2). Cations other than $Ca^{2+}$ also appear to affect histaminopexy. Serum $Mg^{2+}$ levels in asthmatics are low during attacks but normal while patients are free of symptoms (3); magnesium sulfate given intravenously to severe asthmatics gives relief for 18–20 hr (3). Injections of magnesium chloride restore histaminopexy in adrenalectomized and ovariectomized rats for up to 3 months, an effect similar to that of $Ca^{2+}$ but longer lasting (4). In contrast to the divalent alkaline earth cations, the monovalent cation $K^+$ appears to inhibit histaminopexy (2, 5–7).

Previously (8), histamine was found to bind to a plasma peptide, termed peptide H, through the formation of a $Ca^{2+}$–histamine complex. Apparently, only one extensive investigation of histamine–alkaline earth complexes has been reported. Chawla (9) measured association and other thermodynamic constants for $Be^{2+}$, $Ca^{2+}$, and $Mg^{2+}$ complexes with histamine, antistine, and similar molecules at several temperatures. Several studies determined association constants for histamine complexes with various other metals (10–12), and the method of pH titration generally has been used. For accurate results, this technique requires extensive experimental precautions such as those used by Chawla (9).

This report describes the determination of histamine–cation constants at constant pH, using a cation selective electrode. The purpose of this investigation was to deter-