Comparative Pharmacokinetics of Quinidine and Its *O*-Desmethyl Metabolite in Rabbits

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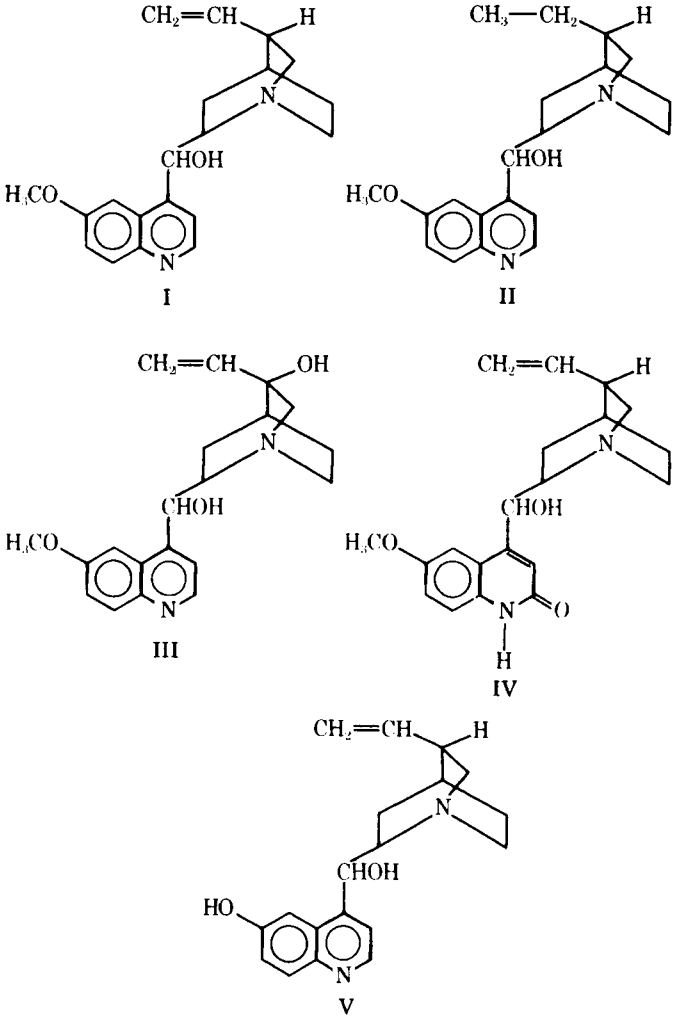
Abstract □ The pharmacokinetics of quinidine and the *O*-desmethyl metabolite 6′-hydroxycinchonine were studied in rabbits. After intravenous bolus injections of equimolar doses, the blood concentration–time curves of each agent declined biexponentially, which was characteristic of a two-compartment open model. A significant difference between quinidine and 6′-hydroxycinchonine was observed for the following parameters: $t_{1/2\alpha}$, β, $t_{1/2\beta}$, k_{12}, Vd_β, and the intercompartmental distribution ratio, k_{12}/k_{21}. The results showed that the distribution of the metabolite was slower than quinidine and suggested that it *also* was less extensive. The Vd_β value for 6′-hydroxycinchonine was approximately one-half of the value observed for quinidine, and its k_{12}/k_{21} ratio was about one-fourth of the quinidine value. The terminal half-lives for quinidine and the metabolite were 132.4 ± 27.1 and 65.4 ± 34.4 min, respectively. Total body clearance was similar for both compounds. The findings of this study could be explained by the greater polarity or greater water solubility of 6′-hydroxycinchonine. The limited data in humans coupled with the results of this investigation suggest that, although it is intrinsically active, the *O*-demethylated metabolite probably contributes little to the antiarrhythmic effects seen after quinidine administration in humans.

Keyphrases □ Quinidine—and its *O*-desmethyl metabolite, comparison of pharmacokinetics in rabbits □ Pharmacokinetics—comparison of quinidine and its *O*-desmethyl metabolite, rabbits □ Antiarrhythmic agents—quinidine and its *O*-desmethyl metabolite, pharmacokinetics, rabbits

6′-Hydroxycinchonine (*O*-desmethylquinidine, V) is a newly reported metabolite of quinidine (I) (1, 2). Nwangwu *et al.* (3, 4) reported that the antiarrhythmic potency of V was equivalent to that of I when tested against aconitine-induced arrhythmias in mice. They also suggested that V was less toxic than I. Drayer *et al.* (1, 2) showed that V possessed ~60% of the antiarrhythmic activity exhibited by quinidine using a chloroform- and hypoxia-induced ventricular fibrillation animal model.

Quinidine metabolites such as 2′-oxoquinidinone (IV), 3-hydroxyquinidine (III), and V are believed to contribute to the antiarrhythmic effects seen after the administration of I in humans[1] (5), but they have not been tested directly for such activity.

The purpose of this study was to investigate the comparative pharmacokinetics of I and V in rabbits. This information could provide important insight into the behavior of this metabolite relative to quinidine in humans.



EXPERIMENTAL

Materials—Restrained, male New Zealand White rabbits[2], 4–5 kg, were used.

Quinidine base, free of the dihydro analog (II), was obtained as described previously (6) and was used to prepare a parenteral solution of quinidine sulfate containing 20 mg (0.062 mmole) of quinidine base/ml of solution. An injectable solution containing 19.14 mg (0.062 mmole) of V[3]/ml was prepared fresh from the dihydrochloride salt just before individual experiments. Chromatographic analysis of V revealed 8% of a substance that was presumed to be its dihydro derivative since this material also was prepared from commercial quinidine sulfate powder[4]. Because it did not interfere with the assay for V, no attempt was made to remove this minor impurity.

Methods—To give the intravenous bolus injections and to withdraw blood samples for drug and metabolite analysis, a 25-gauge vein infusion set with a winged adapter[5] was placed in a marginal ear vein and secured to the external ear with adhesive tape. After a control blood specimen was obtained, 1 ml of drug solution was given as an intravenous bolus dose. Complete administration of each dose was achieved by flushing the contents of the infusion set with ~0.8 ml of heparinized normal saline (5 units/ml). Blood specimens of 0.5 ml for the analysis of I and V were collected in heparinized tubes[6] at 5, 10, 15, 30, 45, and 60 min and thereafter at 0.5-hr intervals for up to 3–4 hr after the dose. The blood samples were stored at −20° until they were assayed.

Blood concentrations of V were determined by the following high-

[1] B. S. Dzindzio, W. M. Vosik, and C. T. Ueda, unpublished data.
[2] Earl B. Daubert, Breeding and Laboratory Rabbits, Omaha, Neb.
[3] Prepared by Dr. Laverne D. Small.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Miniset 2C0075, Travenol Laboratories, Deerfield, Ill.
[6] Becton-Dickinson, Rutherford, N.J.

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performance liquid chromatographic procedure. After the previously frozen samples were thawed, 0.5 ml of whole blood was diluted in a 15-ml culture tube with 0.5 ml of distilled water and 0.5 ml of 0.2 *M* phosphate buffer (pH 7.4), containing 40 ng of dihydroquinidine (II) as the internal standard. Following the addition of 100 μl of 95% ethanol to this mixture, the sample was vortexed[7] and extracted with 4 ml of benzene by shaking for 2 hr on a reciprocating shaker[8] at room temperature.

The benzene extract was separated by centrifugation, and the upper organic layer was carefully removed by pipet and transferred to a 5-ml conical tube. The benzene extract, to which was added 100 μl of absolute alcohol, then was evaporated to dryness at room temperature under a nitrogen stream. The residue was reconstituted with 25 μl of the mobile phase, and an aliquot was injected into a preconditioned liquid chromatograph.

The concentrations of V in the unknown samples were determined from standard calibration curves prepared with the experimental specimens. In the concentration range of 0.5–10 μg/ml for V, the peak height ratio of V to the internal standard was linearly related to the metabolite concentration. The best-fitting equations for the standard curves were obtained by linear regression analysis.

The liquid chromatograph[9] was equipped with a loop injector[10], a μBondapak C_{18} reversed-phase column[11] (30 cm × 3.9 mm i.d.), and a fluorescence detector[12]. The mobile phase, which was filtered and degassed before use, consisted of 15% (v/v) acetonitrile[13] in a 2.5% (v/v) solution of acetic acid and was pumped at a flow rate of 2 ml/min (about 1800 psi). The column eluate was monitored with an excitation wavelength of 315 nm and a KV 418 emission cutoff filter. The detector sensitivity setting was 0.5–1.0 μamp full scale with a time constant set at 4 sec and a chart speed[14] of 0.5 cm/min.

As shown in Fig. 1, with the chromatographic conditions used, V and the internal standard (II) were readily separable with elution times of ~2 and 4 min, respectively. The minor impurity, presumed to be the dihydro analog of V, eluted about midway between the metabolite and internal standard peaks. Peak c in Fig. 1 was an unknown benzene-extractable substance in rabbit blood.

Blood quinidine concentrations were determined with the TLC–fluorometric procedure described previously (7).

After the quinidine and V bolus doses, the blood concentration–time curves for both agents declined biexponentially (Fig. 2). Therefore, the data were fitted using the least-squares computer program NONLIN (8) and a digital computer to:

$$C_b = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

where C_b is the concentration of alkaloid in the blood at time t, A and B are ordinate intercept constants, and α and β are first-order disposition rate constants. Each value was weighted by the reciprocal of the concentration squared. Initial parameter estimates were obtained with the CSTRIP computer program (9).

Equation 1 describes the two-compartment open model shown in Scheme I. This model was used to characterize the disposition of I and V in rabbits. The pharmacokinetic parameters were derived as reported previously (10, 11). The Student t test was used to evaluate the significance of the observed difference.

RESULTS AND DISCUSSION

As shown in Fig. 2 for a representative animal, the blood concentration–time curves for I and V declined biexponentially after intravenous injection of equimolar doses of the two agents. The pharmacokinetic constants derived for each compound are presented in Table I. Three rabbits were given both I and V. The remaining animals received only one agent.

A significant difference between quinidine and the *O*-demethylated metabolite was observed for the following parameters: $t_{1/2\alpha}$, β, $t_{1/2\beta}$, k_{12} (the first-order rate constant for movement of the drug from the central to the peripheral compartment), $V_d\beta$ (the apparent volume of distribution), and k_{12}/k_{21} (the intercompartmental distribution ratio). No difference was observed in the remaining parameters between the two

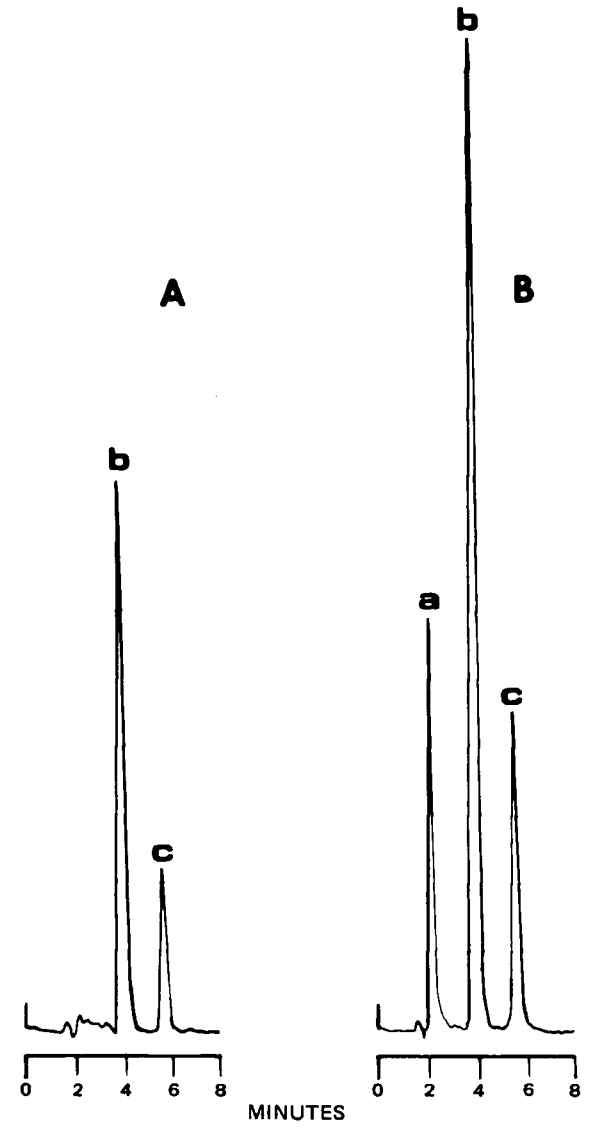
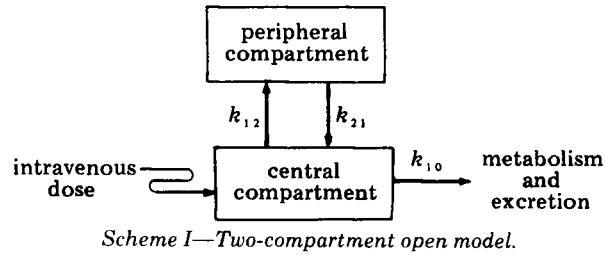


Figure 1—*Liquid chromatograms of blank blood (A) containing the internal standard (II) (b) and a blood sample (B) containing V (a) and II (b). Peak c is an unknown element extracted from rabbit blood.*

compounds; I and V exhibited similar total body clearance rates.

Compared to quinidine, the α or distribution half-life for the metabolite was longer (2.9 *versus* 1.7 min), indicating that it distributed more slowly in the body. The smaller k_{12} value for V (0.134 *versus* 0.226 min^{-1}) agreed with this finding. Furthermore, the distribution of V did not appear to be as extensive as I. The apparent volume of distribution of V of 1.56 ± 0.92 liters/kg was about one-half of the distribution volume for I, which was 3.26 ± 1.11 liters/kg. However, the central compartment volumes for both compounds were of similar size. Thus, the volume of the peripheral or tissue compartment was smaller for the metabolite.

The observed k_{12}/k_{21} ratio, an intercompartmental distribution coefficient, was substantially smaller for the *O*-demethylated metabolite compared to quinidine (4.65 *versus* 17.08). Since the blood flow between the two compartments probably was the same for both agents, the dif-



Scheme I—Two-compartment open model.

[7] Vortex-Genie, Scientific Industries, Springfield, Mass.
[8] Eberbach Corp., Ann Arbor, Mich.
[9] Series 2, model 2/2, Perkin-Elmer, Norwalk, Conn.
[10] Model 7105, Rheodyne, Berkeley, Calif.
[11] Waters Associates, Milford, Mass.
[12] Model FS-970, Schoeffel Instrument Corp., Westwood, N.J.
[13] Burdick & Jackson Laboratories, Muskegon, Mich.
[14] Model 23, Perkin-Elmer, Norwalk, Conn.

Table I—Mean Disposition Constants for 6'-Hydroxycinchonine (V) and Quinidine (I) after Intravenous Bolus Injections in Rabbits (±*SD*)

Parameter	V[a]	I[b]	*t* Value	Significance Level
α, min^{-1}	0.290 ± 0.141	0.445 ± 0.143	2.02	$p < 0.10$
$t_{1/2\alpha}$, min	2.86 ± 1.29	1.72 ± 0.62	2.21	$p < 0.05$
β, min^{-1}	0.013 ± 0.006	0.006 ± 0.002	3.17	$p < 0.01$
$t_{1/2\beta}$, min	65.35 ± 34.39	132.44 ± 27.11	4.09	$p < 0.005$
k_{12}, min^{-1}	0.134 ± 0.048	0.226 ± 0.089	2.28	$p < 0.05$
k_{21}, min^{-1}	0.037 ± 0.024	0.019 ± 0.015	1.73	NS[c]
k_{10}, min^{-1}	0.133 ± 0.131	0.207 ± 0.136	1.03	NS
V_c, liters/kg	0.22 ± 0.15	0.20 ± 0.27	0.14	NS
Cl, ml/min/kg	16.84 ± 5.08	16.80 ± 4.51	0.02	NS
$V_d\beta$, liters/kg	1.56 ± 0.92	3.26 ± 1.11	3.04	$p < 0.02$
k_{12}/k_{21}	4.65 ± 3.10	17.08 ± 9.44	3.08	$p < 0.01$
$V_c \times k_{10}$, ml/min/kg	15.5 ± 4.2	17.0 ± 4.8	0.59	NS

[a] $n = 6$. [b] $n = 8$. [c] Not significant.

ferences in the k_{12}/k_{21} ratio between I and V as well as the observed differences in $t_{1/2\alpha}$, k_{12}, and $V_d\beta$ most likely were the results of the greater lipid solubility of I.

The terminal half-life values of 65.4 ± 34.4 and 132.4 ± 27.1 min for V and I, respectively, showed that the metabolite was eliminated more rapidly than the parent drug. This observation is in agreement with the greater polarity or greater water solubility of the *O*-demethylated molecule and indicates that the metabolite would accumulate to a lesser extent than I on repeated dosing of the parent drug (12).

On the basis of its greater polarity, it was anticipated that V would be cleared at a faster rate than I. Therefore, the similarity in total body clearance for V and I was unexpected. Nevertheless, this finding is explained by the following reason. It is widely recognized that metabolism alters the physicochemical properties (*e.g.*, lipid solubility) of a drug molecule, which then leads to changes in its disposition characteristics. For drugs that are converted to more polar metabolites, these biotransformation products would be expected to have smaller volumes of distribution because their ability to cross lipid membranes by diffusion would be diminished. Furthermore, it is reasonable to expect that they would be eliminated more rapidly (*i.e.*, larger β) since they are more water soluble. From the relationship for total body clearance, $Cl = V_d\beta$, it can be seen that a decrease in V_d and an increase in β tend to offset each other and, thus, minimize the anticipated changes in Cl from situations where V_d or β changes alone.

When metabolism involves the conversion to less polar metabolites, a similar situation would be in effect wherein the volumes of distribution and first-order elimination rate constants of the metabolites would increase and decrease, respectively. This situation was observed recently (13) for diethyldithiocarbamate (a metabolite of disulfiram) and its metabolite, methyl diethyldithiocarbamate in dogs. Diethyldithiocarbamate, with a V_d value of 0.16 liter/kg and a β value of 0.205 min^{-1}, is readily soluble in water. Methyl diethyldithiocarbamate (V_d = 2.53 liters/kg and β = 0.014 min^{-1}) is virtually insoluble in water (highly lipid soluble). The observed total body clearances for the respective drug species were 33.1 and 31.9 ml/min/kg.

In this study, the clearance of V and I from the central compartment ($k_{10} \times V_c$) also was similar (16 *versus* 17 ml/min/kg).

In humans, quinidine is eliminated essentially by metabolism (14, 15). The fate of V is not known. Drayer *et al.* (2) detected conjugated and unconjugated V in the urine of four patients receiving quinidine and estimated that both forms accounted for ~1–2% of the quinidine dose. Preliminary findings in this laboratory also suggest that the *O*-demethylation pathway is a minor route for quinidine elimination in humans. These observations, coupled with the results of this study on the disposition and accumulation characteristics of V in rabbits, suggest that, although the *O*-demethylated metabolite possesses intrinsic antiarrhythmic activity (1–4), it probably contributes little to the antiarrhythmic effects seen after the administration of quinidine in humans.

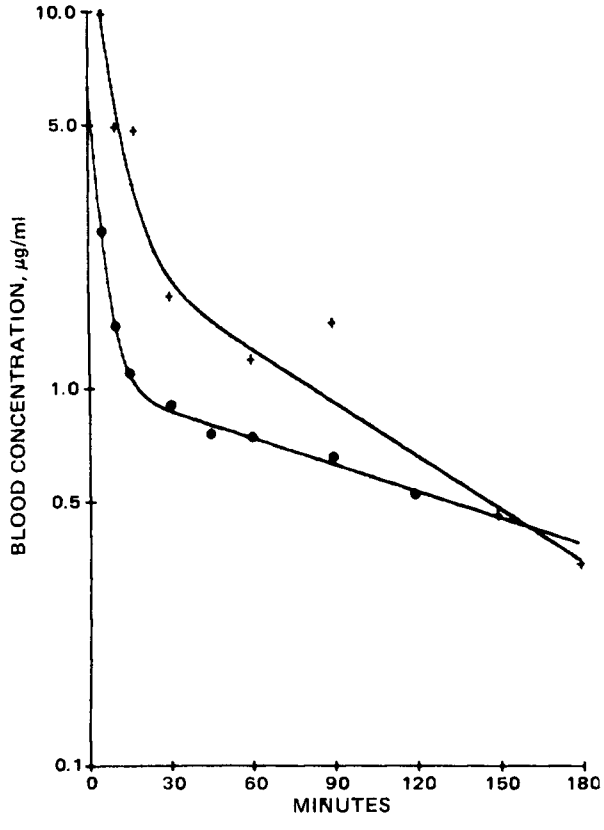


Figure 2—*Computer-simulated curves and experimental data points following intravenous bolus injections of 20 mg of I (●) and 19 mg of V (+) in a single rabbit.*

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Interaction of Povidone with Aromatic Compounds I: Evaluation of Complex Formation by Factorial Analysis

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Abstract □ In a study of complex formation between macromolecules and small ligands such as drugs, it appeared that the association constants must be calculated with more care (*i.e.*, after a thorough investigation of the influencing parameters such as buffer composition, ionic strength, and temperature) to allow meaningful interpretations of the phenomena. For this purpose, factorial analysis seems to be the method of choice; it offers the advantage of evaluating the influence of several variables and their interactions at the same time with a minimum of experiments. The method was applied to the association of povidone with two ligands, salicylic acid and benzoic acid. Parameters such as buffer composition and ionic strength, which affect binding, could be distinguished. Especially at pH 7.00, a great positive influence of buffer ions (phosphate buffer) and a relative positive interaction between temperature and ionic strength were noted. Knowledge of the influences of these parameters allowed comparison of the effects of the functional groups attached to the ligand molecules, as well as their degree of dissociation, on adsorption to permit more meaningful interpretation of thermodynamic constants.

Keyphrases □ Salicylic acid—binding to povidone, effects of temperature, buffer composition, and ionic strength □ Benzoic acid—binding to povidone, effects of temperature, buffer composition, and ionic strength □ Povidone—complex formation with salicylic acid and benzoic acid, effects of temperature, buffer composition, and ionic strength □ Complexation—povidone with salicylic acid and benzoic acid, effects of temperature, buffer composition, and ionic strength

The binding of various drugs with macromolecules in aqueous solution has been reviewed (1–3). Vallner (3) showed the discrepancies among the results obtained by several investigators and cited the need for the calculation of the association constants in a more meaningful manner. These discrepancies often are due to the uncontrolled influence of variables such as buffer composition, pH, ionic strength, and temperature. There have been few reports concerning the influence of buffer ions on the binding onto macromolecules (4, 5). The parameters usually are studied in the classical manner, *i.e.*, by successively changing one parameter at a time. However, as demonstrated previously (6), an accurate interpretation of the individual effects of the variables or their interactions is not possible with that approach.

Factorial analysis offers the possibility of evaluating the influence of individual variables and their interactions at the same time with a minimum of experiments. This method seems to be a first step for a thorough study of complex formation. Once the influence of these parameters is known, comparison of the effects of the functional groups attached to the ligands and their degree of dissociation on adsorption is possible, and the association constants can be interpreted more meaningfully. The application of factorial analysis to the binding of two simple ligands, benzoic acid and salicylic acid, onto povidone demonstrates the advantages and possibilities of this method in the study of complex formation.

EXPERIMENTAL

Reagents—Povidone[1] with a molecular weight of 700,000 was used as the macromolecule and was oven dried at 50° until a constant weight was reached. Salicylic acid[2] (p.a.) and benzoic acid[3] (p.a.) were used without further purification.

Buffer solutions were used at pH 7.00 (phosphate buffer) and at pH values equal to the pKa value of the two cosolutes (McIllvaine buffers) plus and minus 0.80; the solutions were brought to a determined ionic strength with sodium chloride. To control the influence of buffer ions, two kinds of buffer solutions were used; the normal buffers are denoted by 1 and those with half of the normal capacity are indicated as 0.5. The pH of the solutions at 25 and 50° was controlled with a potentiometric pH measurement[4] and adjusted if necessary.

Methods—The solutions containing the ligand and povidone were prepared in the respective buffers and allowed to stand overnight to attain equilibrium. Ultrafiltration[5] was performed, using a membrane of regenerated cellulose with a claimed cutoff value of ~10,000 mol. wt. units. Compressed nitrogen (4 kg/cm^2) was used, and the filtration was performed under continuous stirring of the sample solution to avoid accumulation of the macromolecule at the membrane–solution interface (7).

The concentration of unbound ligand in the filtrate was assayed spectrophotometrically. Corrections were made for membrane adsorption effects, and the concentration of bound ligand was calculated. The spectrophotometric measurements were performed with a double-beam spectrophotometer[6] at 296 nm for salicylic acid and at 224 nm for benzoic acid.

Calculations—Complex formation can be studied as a function of either macromolecule concentration or ligand concentration. In the first method, the relative tendencies of several ligands to form complexes are expressed as the ratio of the total ligand concentration, T, to the con-

[1] Polyvinylpyrrolidone (Kollidon K90), BASF, Brussels, Belgium.
[2] Merck.
[3] U.C.B.
[4] Radiometer, Copenhagen, Denmark.
[5] Amicon model 52.
[6] Perkin-Elmer model 124.

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