Utilization of Model Compounds to Evaluate Effects of Slight Chemical Modifications on Their Distribution Pharmacokinetic Parameters in Rats and Mechanisms Inferred for Their Transmembrane Transport

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Abstract □ The pharmacokinetics of each of the model compounds benzoylformic acid (I), *p*-methylbenzoylformic acid (II), *p*-ethylbenzoylformic acid (III), D-(−)-mandelic acid (IV), D-(−)-*p*-methylmandelic acid (V), D-(−)-*p*-ethylmandelic acid (VI), and D-(−)-*p*-isopropylmandelic acid (VII) were studied in rats to determine the influence of slight chemical modifications of the compounds on their distribution pharmacokinetic parameters in rats. The effects of the specific chemical modifications considered were those of the >CHOH group of IV against the >C=O group of I, the *para*-alkylation of I and IV, and the branched alkyl group (isopropyl) against the straight chain alkyl groups of the homologs of IV. While the disappearance of I from the blood was describable by the three-compartment open model, that of IV was describable by the two-compartment open model. The apparent volume of distribution of the central compartment (V_1) for IV was smaller than that for I, but the volume of the peripheral compartment (V_2) for IV was greater than that ($V_2 + V_3$) for I. The disappearance of V, VI, and VII from the blood was also describable by the two-compartment open model, but the apparent V_1 and V_2 for these compounds were lower than those for the parent compound, IV. However, the disappearance of II and III from the blood was describable by a one-compartment open model. Evaluation of the appropriate distribution pharmacokinetic parameters suggested that the peripheral compartment for the anions of these compounds consisted of moderately perfused tissues and that the transmembrane transport of these organic anions between the central and peripheral compartments occurs by diffusion mainly through the aqueous membrane pores, which are lined with polar portions of the membrane proteins and/or phospholipids. The possible increased hydrophobic bonding between the alkyl groups of these compounds and the hydrophobic groups of the proteins and/or phospholipids of the membrane pores is implicated to decrease the distribution of the *para*-alkylated homologs into the peripheral compartments and, consequently, diminish the volumes of their peripheral compartments. The heteroporosity of the membranes of the tissues of the central compartment is proposed as the reason for the diminished volume of the central compartment for V and VI as compared to that of IV or VII.

Keyphrases □ Pharmacokinetics—distribution parameters, use of model compounds to evaluate effects of chemical modifications, mechanisms of transmembrane transport, rats □ Distribution—model compounds, effects of chemical modifications, pharmacokinetic parameters, transport mechanisms, rats □ Transport—model compounds, effects of chemical modifications, distribution pharmacokinetic parameters, rats

It is well recognized that the extent of pharmacological response of a drug depends not only on the drug–receptor interaction but also on the concentration of the drug at the site of action containing the receptor(s). Therefore, it is common practice to synthesize and use the analogs or homologs of drugs to modify their biological activities. Although theories have been suggested concerning the drug structure–activity relationship on the basis of biological effects, such theories are not complete without considering the influence on the pharmacological actions of the rates of distribution of drugs between plasma and tissues where the site(s) of action may reside. However, the rates of distribution of drugs at the site(s) of action are governed by several processes, namely, binding of drugs to plasma proteins, metabolism of drugs, renal and nonrenal excretion, and renal tubular secretion of drugs, all of which occur simultaneously.

In recent years, studies have been performed to compare the effects of substituent groups of analogs or homologs of drugs on their pharmacokinetic parameters to determine the effect of specific substituent groups on the specific pharmacokinetic parameters of the compounds. However, such efforts do not appear to be too successful, mainly because the determination of the pharmacokinetic parameters is complicated due to the differences of the homologs or analogs in their extent of binding to plasma (1–6), pKa values, rates of metabolism (7), and partition coefficients between aqueous and lipid phases within the body (4, 7).

According to current concepts, biological membranes are composed mainly of proteins and phospholipids; the proportions of these building blocks are not identical for the membranes of all organs. Several mechanisms for the transmembrane transport of solutes (mainly inorganic electrolytes and organic nonelectrolytes) have been proposed, including diffusion of permeant molecules by dissolving in the membrane; diffusion of permeant molecules through the aqueous pores, which are lined with polar portions of the proteins and/or phospholipids as carrier(s); and active transport, which may involve enzymes and adenosine triphosphatase (8). Therefore, depending upon the organ of permeation and mechanism of transport of solutes through the membrane of such organ, the differences in the rates of diffusion of the compounds can be expected.

In view of these various mechanisms for transmembrane transport of solutes and their interaction with components of the membrane through various forces (8) (electrostatic interactions, hydrogen bonding, and hydrophobic bonding), it is apparent that the effects of substituent groups of homologs or analogs of drugs on the distribution pharmacokinetic parameters cannot be determined unless the effects of the various processes (protein binding, metabolism, and renal tubular reabsorption) to which the drug is subjected simultaneously in the body are minimal. Consequently, studies with several drugs and their

homologs and/or analogs were unable to determine exclusively the effects of substituents on the distribution pharmacokinetic parameters (1–7).

In recent years, D-(−)-mandelic acid and several of its *para*-alkylated homologs have been shown to possess ideal properties by virtue of which they are neither metabolized nor reabsorbed from the renal tubules of rats and are negligibly bound to plasma proteins (9–12). Since these compounds have low pKa values, they remain completely ionized in the body fluids and serve as model organic anions.

Therefore, the purposes of this project were to study the pharmacokinetics of benzoylformic acid (I) and its *para*-alkylated homologs *p*-methylbenzoylformic acid (II) and *p*-ethylbenzoylformic acid (III) as well as D-(−)-mandelic acid (IV) and its *para*-alkylated homologs D-(−)-*p*-methylmandelic acid (V), D-(−)-*p*-ethylmandelic acid (VI), and D-(−)-*p*-isopropylmandelic acid (VII) from blood level data in rats and to determine the effects of slight chemical variations on their distribution pharmacokinetic parameters. The effects of the specific chemical modifications considered were those of the >CHOH group of IV against the >C=O group of I, the *para*-alkylation of I and IV, and the branched alkyl group (isopropyl) of VII against the straight chain alkyl groups of V, VI, and D-(−)-*p*-*n*-propylmandelic acid (13).

An additional purpose of this project was to utilize the difference observed in the pharmacokinetic parameters of the compounds to reveal the possible mechanisms for their transmembrane transport between central and peripheral compartments of the body at a molecular level by considering the possible electrostatic, hydrogen bonding, and hydrophobic interactions between the permeant molecules and certain components of the membranes.

EXPERIMENTAL

Materials—The following were used: D-(−)-mandelic acid[1], mp 132–133°, $[\alpha]_D^{25}$ −154°; D-(−)-*p*-methylmandelic acid[2], mp 130–131°, $[\alpha]_D^{25}$ −159°; D-(−)-*p*-ethylmandelic acid[2], mp 119–120°, $[\alpha]_D^{25}$ −120°; D-(−)-*p*-isopropylmandelic acid[3], mp 159–160°, $[\alpha]_D^{25}$ −129°; benzoylformic acid[1], mp 62–64°; *p*-methylbenzoylformic acid, mp 97–99°, synthesized by the method of Kindler *et al.* (14); *p*-ethylbenzoylformic acid, mp 65–67°, also synthesized by the method of Kindler *et al.* (14); and DL-tropic acid[1], mp 118–119°.

Apparatus—Compounds I, IV, and their respective *para*-alkylated homologs present in rat blood were analyzed by GLC[4] using a hydrogen-flame detector.

Methodology—Approximately 250 Sprague–Dawley male rats, weighing between 170 and 230 g (a majority weighed ~200 g), were used. Blood levels of the compounds were determined in eight to 10 rats on a given day. Food was withheld from the rats 12–14 hr prior to their use in the study as well as during the study.

Five milliliters of normal saline was injected intraperitoneally into each rat 20 min prior to intravenous administration of the compound(s) in order to be consistent with the procedure followed previously (9–13) in urinary excretion studies so that the pharmacokinetic parameters obtained from the blood level studies can be compared with those obtained from the urinary excretion studies. The rat was anesthetized with ether for less than 1 min for intravenous injection of a fixed dose of the compound contained in 2 ml of isotonic solution. Each injection time was accurately noted.

Only one blood sample was obtained from a given rat upon its decapitation at an appropriate time following administration of the intravenous dose of the compound. The blood samples, which were obtained over 1–100 min for benzoylformic acid and over 1–65 min for the rest of the compounds, were collected in 50-ml beakers previously coated with heparin to prevent coagulation of blood. Each beaker was coated with 0.1 ml (10 units) of heparin. The blood samples were analyzed for the compound on the same day they were collected. The pharmacokinetics of I were studied at the three intravenous dose levels (166.65[5], 249.80, and 333.33 μmoles/kg). The pharmacokinetics of II (152.45 μmoles/kg)[5], III (140.5 μmoles/kg)[5], IV (165.35 μmoles/kg)[5], V (149.6 μmoles/kg)[5], VI (138.25 μmoles/kg)[5], or VII (128.1 μmoles/kg)[5] were studied at a single intravenous dose level. Each compound was injected as its sodium salt.

GLC Analysis—Depending on the anticipated concentration of the compound in a blood sample, an appropriate volume of blood (1–5 ml) was transferred to a 150-ml beaker. The proteins and blood cells were precipitated with a dropwise addition of 5 *N* HCl, with continuous stirring for 10 min with a magnetic stirrer. To extract the compounds from this blood sample into ether, 25 ml of ether was added to the blood sample and stirring was continued for another 15 min. The ethereal layer was then quantitatively transferred to a 60-ml separator, and it was extracted with 15–20 ml of 5 *N* sodium hydroxide solution to recover completely the compound in the alkaline aqueous phase as its sodium salt; the ethereal layer was discarded.

The residue of each precipitated blood sample remaining in the 150-ml beaker after the first ether extraction was further extracted with two additional 20-ml volumes of ether for I and its *para*-alkylated homologs and with three additional 20-ml volumes of ether for IV and its *para*-alkylated homologs. The compound was recovered from these ethereal phases using the alkaline solution employed in the first extraction. The alkaline solution was then acidified with 5 *N* HCl and extracted twice with ether for I and its *para*-alkylated homologs and three times for IV and its *para*-alkylated homologs, using 30 ml of ether each time.

The ethereal layer was collected in a 100-ml beaker and the ether was evaporated by transferring the ethereal layer in portions to a 20-ml graduated tube. The ether was evaporated from the test tube on a water bath maintained at 42°. The residue of the compound thus obtained in the test tube was treated with sufficient amount of ethereal diazomethane to convert the compound to its methyl ester. Excess ether and diazomethane were removed from the tube on a water bath at 42°. A sufficient quantity of absolute methanol was added to the residue to dissolve the compound and to adjust the volume to 1 ml. The methanolic solution thus prepared was ready for GLC analysis.

An appropriate volume (generally 20 μl) of the methanolic solution was injected onto the GLC column. The conditions employed for the GLC analysis and the column used are described in Table I.

To determine accurately the amount of a compound (*i.e.*, I or one of its homologs or IV or one of its homologs) present in the blood samples, a calibration curve for the compound was prepared in the following manner. The solutions of the compound were prepared in the blood obtained from generally three rats, which were not previously treated with any compound. To each 2-ml volume of blood, a known quantity of the compound was added. Then each solution was treated to obtain the methyl ester of the compound according to the procedure described previously. The known quantities of the compound added to 2-ml blood volumes were those obtained from the stock solution prepared on the same day for injecting the rats.

The calibration curve for the compound was also prepared in this manner by using 5 ml of blood, instead of 2 ml, to determine the influence of blood volume on the quantitative recovery of the compound (Fig. 1). The area under each chromatogram was calculated by the trapezoidal rule and plotted against the corresponding amount of the compound (Fig. 1). With reference to such a calibration curve, prepared separately for each study, the amount of the compound present in each blood sample was calculated.

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Previously synthesized (Ref. 11).
[3] Previously synthesized (Ref. 12).
[4] F&M 810R gas chromatograph.

[5] These doses represent 25 mg/kg.

Table I—Conditions Employed for the GLC Analysis and Retention Time Observed for the Methyl Esters of the Acids

Compound	Column Used	Oven Temperature	Detector and Injection Port Temperature	Helium Flow Rate, ml/min	Retention Time, min
I	3% E.G.S.[a]	150°	200°	40	5.2
II	3% E.G.S.[a]	150°	200°	40	8.6
III	3% E.G.S.[a]	160°	210°	40	6.0
IV	5% E.G.S.[a]	165°	210°	60	5.8
V	15% GE-SE 30[b]	180°	210°	40	5.8
VI	15% GE-SE 30[b]	180°	210°	40	7.2
VII	5% E.G.S.[a]	165°	210°	40	12.2

[a] Ethylene glycol succinate on Diatoport S (80–100 mesh) packed in a 1.2-m (4-ft) long and 0.6-cm (0.25-in.) diameter copper column. [b] GE-SE 30 silicone gum rubber on Diatoport S (80–100 mesh) packed in a 1.2-m (4-ft) long and 0.6-cm (0.25-in.) diameter copper column.

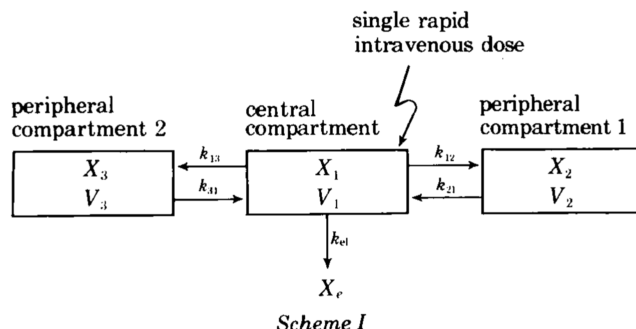
Determination of Dissociation Constants and Apparent Partition Coefficients of Compounds and Their Binding to Whole Rat Blood as well as Plasma Proteins—These determinations for I and its *para*-alkylated homologs were carried out by the methods previously described for IV and its several homologs (9–12).

RESULTS

Selection of Compounds—Selection of the compounds for the present study was based on the following ideal properties:

1. From the overall urinary recovery (Table II) of the administered intravenous doses of I–III, it was demonstrated that virtually 100% of the administered dose of each compound was recovered in the unchanged form, indicating negligible metabolism in rats. The fact that the intravenous dose of IV–VII is also completely recovered in the urine in the unchanged form was already reported (9–12).
2. The pKa values determined for I, II, and III are 3.2, 3.3, and 3.3, respectively; those reported for IV, V, VI, and VII are 3.3, 3.4, 3.4, and 3.6, respectively. Therefore, these compounds should remain in the ionized form in the biological fluids.
3. The partition coefficients ($C_{organic}/C_{buffer}$) determined for these compounds, according to the procedure previously described (9), were zero. Therefore, it was assumed that the reabsorption of the compounds in the renal tubules is negligible.
4. From the equilibrium dialysis studies, it was noted that at the equilibrium concentrations of 1.07 mg/4 ml of I, 0.51 mg/4 ml of II, and 1.03 mg/4 ml of III in the blood as well as in plasma, the extent of binding observed was 13.5, 4, and 4%, respectively. If, as generally believed (15), the plasma protein–drug complex is assumed to dissociate rapidly at the secretion site, the effect of 13.5% binding of I to plasma proteins can be expected to exert no pronounced effect on the biological half-life of the compound. Therefore, for practical purposes, this extent of binding of I as well as of II and III was considered negligible. The binding of IV–VII to plasma proteins also is negligible (9–12).

Determination of Pharmacokinetic Parameters—*Pharmacokinetics of I–III*—The pharmacokinetics of I were studied at three intravenous doses to determine if there were any apparent dose-dependent kinetics in the 166.65–333.33-μmole/kg dosage range. The typical blood level data obtained are presented in Fig. 2. Feathering of the semilogarithmic plots of the data obtained at each dose indicated that the decline in blood levels of the compound can best be described to occur triexponentially rather than biexponentially (Fig. 3). Therefore, these data were analyzed according to the three-compartment open model (Scheme I) with elimination occurring from the central compartment. The elimination process consisted of only urinary excretion, as demonstrated from the overall urinary excretion studies.



Scheme I

Table II—Overall Urinary Excretion Recovery Observed following Intravenous Administration of I–III to Rats

Compound	Dose per Rat, mg	Number of Studies	Intact Compound Recovered, mg
I	5	6	4.79–4.92
	10	4	9.63–10.00
II	5	3	4.92–4.96
	10	3	8.76–9.85
III	1	1	1.02
	3	1	2.73
	5	1	4.85
	10	3	8.90–9.85

In Scheme I, X_1, X_2, and X_3 are the amounts of the compound in the central compartment, peripheral compartment 1, and peripheral compartment 2, respectively, at time *t*; V_1, V_2, and V_3 are the apparent volumes of the central compartment, peripheral compartment 1, and peripheral compartment 2, respectively; X_e is the cumulative amount of the compound excreted in the urine up to time *t*; k_{el} is the apparent first-order rate constant of elimination of the compound; and k_{12}, k_{21}, k_{13}, and k_{31} are the apparent first-order rate constants for the transfer of the compound from one compartment to the other.

The general solution for the three-compartment open model was described (16) as:

$$C_b = C_1 e^{-\alpha t} + C_2 e^{-\beta t} + C_3 e^{-\gamma t} \quad \text{(Eq. 1)}$$

where C_b is the concentration of the compound in the blood at time *t*. The blood data were plotted on semilogarithmic paper (Fig. 2), and preliminary estimates for the intercepts (C_1, C_2, and C_3) and slopes ($-\alpha/2.303$, $-\beta/2.303$, and $-\gamma/2.303$) were obtained by using appropriate equations (16).

By using these initial estimates, the blood level data were fitted to a three-compartment open model according to the NONLIN least-squares program (17). The excellent fit of the data was indicated by the high coefficient of determination and the correlation

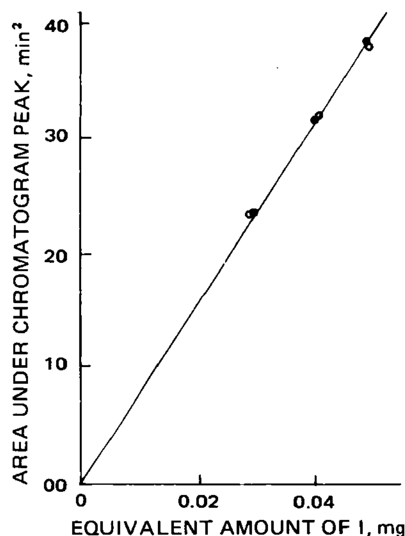


Figure 1—*Standard curve prepared for I contained in 2 (●) and 5 (○) ml of blood, indicating negligible influence of blood volume on the recovery of the compound from the blood.*

Table III—Pharmacokinetic Parameters Estimated and Derived for the Three-Compartment Open Model by Nonlinear Least-Squares Fitting (NONLIN) of Whole Blood Concentration Data Obtained for I in Rats

Pharmacokinetic Parameters	Intravenous Dose (166.65 μmoles/kg)	Intravenous Dose (249.80 μmoles/kg)	Intravenous Dose (333.33 μmoles/kg)	Average for Three Studies
Estimated Parameters				
k_{12}, min^{-1}	0.0518	0.0785	0.0432	0.0578
	(0.199)[a]	(0.03)	(0.076)	
k_{21}, min^{-1}	0.1707	0.1108	0.1762	0.1525
	(0.177)	(0.038)	(0.175)	
k_{13}, min^{-1}	0.1126	0.0770	0.1313	0.1069
	(0.156)	(0.023)	(0.066)	
k_{31}, min^{-1}	0.0617	0.0383	0.0551	0.0518
	(0.090)	(0.021)	(0.020)	
k_{el}, min^{-1}	0.1037	0.1003	0.1035	0.1025
	(0.025)	(0.014)	(0.005)	
α, min^{-1}	0.3436	0.3103	0.3471	0.3336
β, min^{-1}	0.1328	0.0769	0.1416	0.1171
γ, min^{-1}	0.0239	0.0180	0.0205	0.0205
V_1, ml/kg	242.95	220.85	218.45	227.40
	(10.30)	(7.20)	(9.50)	
Derived Parameters				
V_2, ml/kg	85.60	186.80	60.65	110.00
V_3, ml/kg	723.75	825.45	829.10	792.75
Cb^0, μmoles/ml	0.6859	1.1310	1.4280	
f_c	0.2290	0.1800	0.1980	0.2020
k_{12}/k_{21}	0.3030	0.7080	0.2450	0.4187
k_{13}/k_{31}	1.8270	1.9930	2.3810	2.0670
$t_{1/2\gamma}$, min	29.00	38.50	33.80	33.78
Measures of Fit				
Coefficient of determination (r^2)	0.996	0.998	0.991	
Correlation coefficient	0.995	0.998	0.992	

[a] The number in parentheses refers to the standard deviation of the corresponding parameter.

coefficient (Table III). The volumes of the central compartment, peripheral compartment 1, and peripheral compartment 2 were calculated, using appropriate equations. The volume of the central compartment (V_1) refers to the volume of the blood and interstitial fluids that are readily accessible to the compound, and V_2 and V_3 refer to the volumes constituted by different tissue fluids.

To determine if I shows any apparent dose-dependent kinetics in the dosage range used, the blood level data of each dose were treated according to the superposition method (18). Accordingly, plots of C_b/intravenous dose *versus* time were prepared for the blood level data obtained at each dosage level. As can be seen in Fig. 3, no apparent dose-dependent kinetics are observed. Therefore, to facilitate the interpretation of the data in the later sections, the appropriate pharmacokinetic parameters obtained at these three dosage levels were averaged (Table III).

The pharmacokinetics of II and III each were studied at only one intravenous dose level (25 mg/kg); there was no specific need to determine their dose-dependent kinetics for this study. Examination of the blood level data obtained for these compounds (Fig. 4) indicated that their disappearance from the blood occurred monoexponentially. Therefore, these data were analyzed according to the one-compartment open model (Scheme II). The elimination process of the compound consisted of urinary excretion only, as demonstrated from the overall urinary excretion studies (Table II).

In Scheme II, X_1 is the amount of the compound in the body at time t, X_e is the cumulative amount of the compound excreted in the urine up to time t, k_{el} is an apparent first-order rate constant for elimination of the compound, and V_1 is an apparent volume of

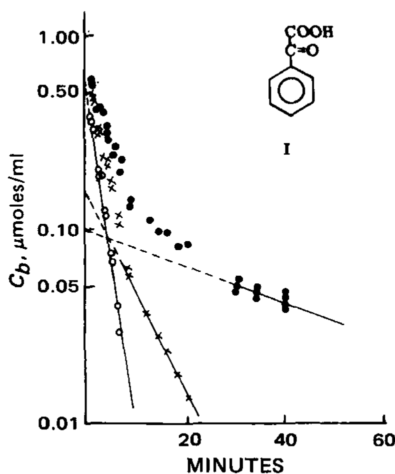


Figure 2—*Plot showing triexponential decline of blood levels of I following administration of a 166.65-μmoles/kg iv dose per rat. (Each data point represents the concentration of the compound noted in a given rat, as explained in the text.) Key:* ●, *experimental data points; and* ×, ○, *data points obtained upon feathering.*

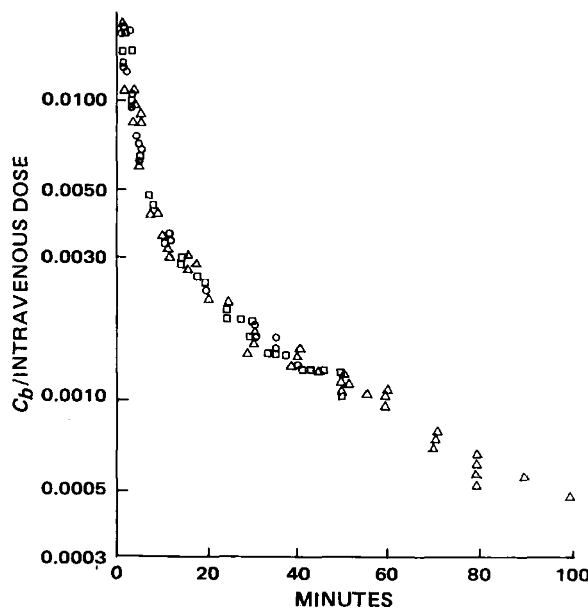
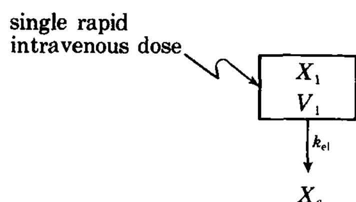


Figure 3—*Superposition plot of C_b/intravenous dose* versus *time, indicating the apparent absence of dose-dependent kinetics for I in the dosage range of 166.65–333.33 μmoles/kg in rats. Key:* ○, *166.65-μmoles/kg iv dose;* □, *249.80-μmoles/kg iv dose; and* △, *333.33-μmoles/kg iv dose.*



Scheme II

distribution for the compound in the body. The blood level data were treated according to the following equation:

$$\log C_b = \log C_b{}^0 - k_{el}t/2.303 \quad \text{(Eq. 2)}$$

where C_b is the concentration of the compound at time t, and $C_b{}^0$ is the concentration of the compound at zero time. The values of $C_b{}^0$ for these compounds were determined from the intercepts obtained by extrapolating the respective least-squares straight lines to zero time (Fig. 4). The volumes of distribution and the biological half-lives were calculated according to the equations V_d = intravenous dose/$C_b{}^0$ and $t_{1/2} = 0.693/k_{el}$. The pharmacokinetic parameters determined are recorded in Table IV.

Pharmacokinetics of IV–VII—Examination of the data obtained for IV (Fig. 5) indicated that the disappearance of the compound from the blood occurs biexponentially. Therefore, these data were treated according to the two-compartment open model (Scheme III), with elimination occurring from the central compartment. The elimination process for the compound consisted of urinary excretion only, as demonstrated by Randinitis *et al.* (9).

In Scheme III, X_1 and X_2 are the amounts of the compounds in the central compartment and the peripheral compartment, respectively, at time t; X_e is the cumulative amount of the compound excreted in the urine up to time t; V_1 and V_2 are the apparent volumes of the central compartment and the peripheral compartment, respectively; k_{el} is the apparent first-order rate constant of elimination of the compound; and k_{12} and k_{21} are the apparent first-order rate constants for the transfer of the compound from one compartment into another. The general solution of this system gives the following equation:

$$C_b = C_1e^{-\alpha t} + C_2e^{-\beta t} \quad \text{(Eq. 3)}$$

where C_b is the concentration of the compound in the blood at time t. Preliminary estimates for the intercepts (C_1 and C_2) and slopes ($-\alpha/2.303$ and $-\beta/2.303$) were obtained by feathering the semilogarithmic plot (Fig. 5). The initial estimates for k_{12}, k_{21}, and k_{el} were obtained using the usual equations (19). By using these initial estimates, the blood level data were fitted to a two-compartment open model according to the NONLIN least-squares program (17). The estimated and derived parameters are presented in Table V. The excellent fit of the data is indicated by the high coefficient of determination and the correlation coefficient (Table V). The volumes of the central compartment (V_1) and the peripheral compartment (V_2) were calculated from V_1 = intravenous dose/$C_b{}^0$ and $V_2 = (k_{12}/k_{21})V_1$, respectively (19). The fraction of the dose of the compounds, $f_c = \beta/k_{el}$, and the $t_{1/2\beta} = 0.693/\beta$ values were calculated.

Table IV—Pharmacokinetic Parameters Determined for II (152.45 µmoles/kg iv) and III (140.50 µmoles/kg iv) from Blood Level Data Obtained in Rats

	II	III
Pharmacokinetic Parameters		
$C_b{}^0$, µmoles/ml	0.6194	1.137
k_{el}, min^{-1}	0.0410	0.133
$t_{1/2}$, min	14.10	5.19
V_d, ml/kg	246.10	123.50
Measures of Fit		
Coefficient of determination (r^2)	0.997	0.996
Correlation coefficient	0.998	0.998

The data obtained for V–VII are presented in Fig. 6. Examination of the blood level data for each compound revealed that the decline in the blood levels of the compounds also occurs biexponentially, as was noted for IV. Therefore, these data were analyzed according to the two-compartment open model system and the initial estimates were determined. By using the initial estimates, the blood level data were fitted to a two-compartment open model according to the NONLIN least-squares program (17). The estimated and derived parameters are presented in Table V. The excellent fit of the data is indicated by the high coefficient of determinations and the correlation coefficients (Table V).

DISCUSSION

Effect of Substitution of Alkyl Groups in the *para*-Position of IV on Their Distribution Pharmacokinetic Parameters—To determine the effect of alkyl group substitution on the distribution pharmacokinetic parameters of IV and its *para*-alkylated homologs, the parameters appropriate for evaluation are V_1, V_2, and k_{12}/k_{21}. Examination of the data presented in Table V reveals a certain trend in the magnitudes of these parameters which relate to the influence of the alkyl groups.

Comparison of V_1 *and* V_2—In general, the volume of the central compartment (V_1) for IV appears to be greater than that for its *para*-alkylated homologs, although the V_1 for VII may be considered very similar to that for IV. The apparent volume of the peripheral compartment (V_2) for IV is greater than that for each of its homologs. Substitution of a methyl group or an ethyl group resulted in a sharp decrease (by a factor of almost 4.5–5) in the volume of distribution of the peripheral compartment, whereas sub-

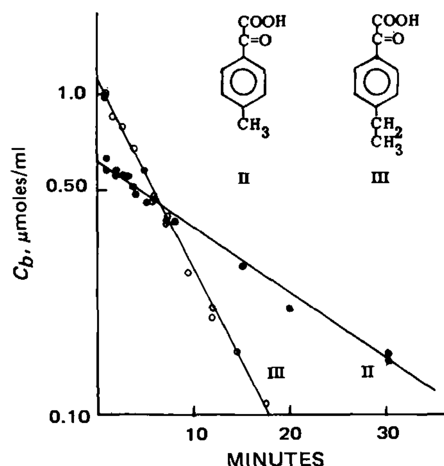


Figure 4—*Plot showing monoexponential decline of blood levels of II (●) and III (○) following administration of a 152.45- and 140.50-µmoles/kg iv dose per rat, respectively. (Each data point represents the concentration of the compound noted in a given rat, as explained in the text.)*

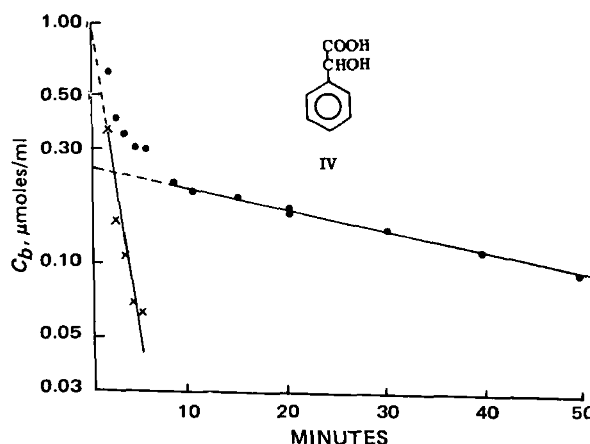


Figure 5—*Plot showing biexponential decline of blood levels of IV following administration of a 164.35-µmoles/kg iv dose per rat. (Each data point represents the concentration of the compound noted in a given rat, as explained in the text.) Key: ●, experimental data points; and ×, data points obtained upon feathering.*

Table V—Pharmacokinetic Parameters Estimated and Derived for the Two-Compartment Open Model by Nonlinear Least-Squares Fitting (NONLIN) of Whole Blood Concentration Data Obtained for IV and Its *para*-Alkylated Homologs in Rats

Pharmacokinetic Parameters	IV (164.35 μmoles/kg)	V (149.60 μmoles/kg)	VI (138.25 μmoles/kg)	VII (128.10 μmoles/kg)
Estimated Parameters				
C_b^0, μmoles/ml	0.8537 (0.049)[a]	0.9070 (0.015)	0.9213 (0.090)	0.6806 (0.038)
k_{12}, min^{-1}	0.3377 (0.036)	0.2107 (0.008)	0.2495 (0.052)	0.1633 (0.028)
k_{21}, min^{-1}	0.0580 (0.003)	0.1597 (0.002)	0.1319 (0.016)	0.0718 (0.006)
k_{el}, min^{-1}	0.1891 (0.016)	0.2102 (0.008)	0.2035 (0.061)	0.1817 (0.038)
α, min^{-1}	0.5667	0.5154	0.5347	0.3826
β, min^{-1}	0.0200	0.0651	0.0502	0.0340
Derived Parameters				
V_1, ml/kg	192.50	164.95	149.90	188.20
V_2, ml/kg	1121.20	217.75	283.60	428.90
k_{12}/k_{21}	5.82	1.32	1.89	2.28
fc	0.106	0.310	0.247	0.187
$t_{1/2\beta}$, min	34.70	10.60	13.80	20.40
Measures of Fit				
Coefficient of determination (r^2)	0.997	1.000	0.998	0.981
Correlation coefficient	0.997	1.000	0.999	0.993

[a] The number in parentheses refers to the standard deviation of the corresponding parameter.

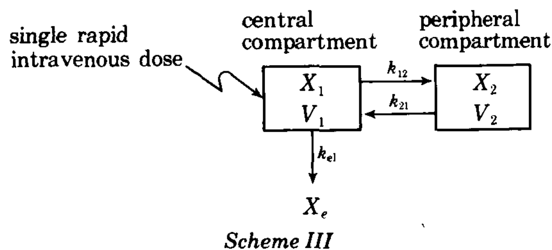
stitution of an isopropyl group resulted in a decrease in the volume of the peripheral compartment by a factor of 2.

It is generally believed that the peripheral compartment consists of less moderately or poorly perfused tissues such as fatty tissues. Therefore, it is argued that if the peripheral compartment in which these compounds are distributed does consist of poorly perfused fatty tissues, then the *para*-alkylated compounds, because of their lipophilic alkyl groups, should distribute into the peripheral compartment to a greater extent than does IV, and each should consequently exhibit higher values of V_2 than those observed for IV. This, however, was not the case in the present study.

Furthermore, since IV and its *para*-alkylated homologs have pKa values in the 3.3–3.8 range, all of these compounds should remain in the systemic body fluids in the anionic form and are, consequently, least soluble in the fatty tissues or poorly perfused tissues. Therefore, it is believed that, for the compounds under discussion, the peripheral compartment consists of moderately perfused tissues and the rate of diffusion of each *para*-alkylated homolog into such tissues is significantly lower than that of IV.

Comparison of Ratios of k_{12}/k_{21}—Although the individual distribution rate constants, k_{12} and k_{21}, are calculated in the present study, it is considered desirable to compare the ratios of these rate constants (k_{12}/k_{21}), rather than the individual rate constants, to determine the effect of alkyl groups of the homologs of IV on their distribution characteristics. The reason for this approach is that one cannot be certain if the values of the forward or backward distribution rate constants are absolute since these rate constants are the result of various diffusion steps involved in a given compartment and these rate constants are calculated from the hybrid parameters of α and β.

The appropriateness of this approach is illustrated by the fact that when the distribution rate constants k_{12} = 0.993 hr^{-1} and k_{21} = 1.99 hr^{-1} determined by Wagner *et al.* (20) for spectinomycin were recalculated for the data by Pfeffer (21), using a different computer program, the values obtained were k_{12} = 0.160 hr^{-1} and k_{21} = 0.345 hr^{-1}. Although the values of k_{12} and k_{21} determined by Wagner *et al.* (20) did not agree with those determined by Pfeffer (21), their ratios of k_{12}/k_{21} were very similar. Likewise, while the values of the distribution rate constants reported for ^{3}H-alphaprodine (22) in the same dog are k_{12} = 0.097 min^{-1} and k_{21} = 0.042 min^{-1} at a 1-mg/kg iv dose, the values reported for these constants are k_{12} = 0.059 min^{-1} and k_{21} = 0.028 min^{-1} at a 2-mg/kg iv dose, indicating the possible differences that can exist in the individual values of the distribution rate constants determined at different doses. However, when the ratios of k_{12}/k_{21} are considered for these two doses, the values are very similar. As seen in Table V, the ratio k_{12}/k_{21} obtained for IV in the present study is almost 3–4 times greater than the ratios for its homologs.



Scheme III

To account for (*a*) the lower values of V_2 noted for V–VII than for IV, (*b*) the slightly lower values of V_1 noted for V and VI than for IV or VII, and (*c*) the lower k_{12}/k_{21} ratios noted for V–VII than for IV, it is desirable that the general structure of biological membranes and the mechanisms for transmembrane transport of solutes be considered. Although the biological membranes are composed mainly of phospholipids and proteins, the proportions of these building blocks are not identical for membranes of all organs.

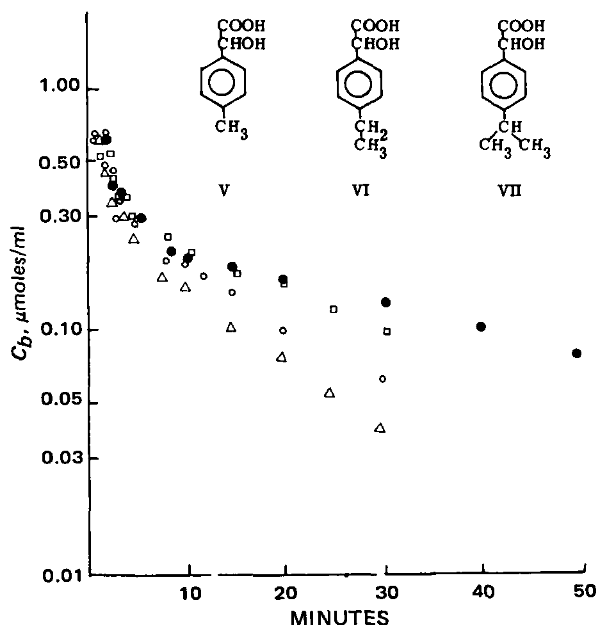


Figure 6—*Plot showing biexponential decline of blood levels for para-alkylated homologs of IV. Key:* ●, *IV;* △, *V;* ○, *VI; and* □, *VII.*

For instance, Aschworth and Green (23) showed that the amounts of phospholipids in the red blood cell ghost, liver plasma, muscle sarcolema, and mitochondrial membrane of liver and mitochondrial membranes of muscles of rats are approximately 61, 42, 28, and 11 mg/100 mg of proteins, respectively. Furthermore, they reported that the membranes of the microsomal liver fractions I, II, and III of rats contain 30, 80, and 35 mg of phospholipids/100 mg of protein, respectively.

For transmembrane transport of solutes, several mechanisms (8) have been proposed including: (*a*) diffusion of permeant molecules by dissolution in the membrane; (*b*) diffusion of permeant molecules through aqueous pores lined with polar portions of the proteins and possibly phospholipids; (*c*) carrier-mediated transport, which may include protein and phospholipid as carriers; and (*d*) active transport, which may involve enzymes and adenosine triphosphatase. Therefore, depending upon the organ of permeation and the mechanism of transport of solute through the membrane of the organ, differences in the rates of diffusion of the compounds of a homologous series can be expected.

Although several studies described the transport of organic nonelectrolytes and the ions of inorganic electrolytes through the membranes of many body tissues or organs, the transmembrane transport of organic electrolytes (ionized forms of acids and bases) has been studied mainly through the renal tubular membrane. Previous studies (9–13) showed that a carrier mechanism is involved in the renal tubular transport of IV and its homologs and that the affinity of VI and D-(−)-*p*-*n*-propylmandelic acid for the carrier is greater than that of IV.

In the present study, however, the transport of V–VII from the central compartment into the peripheral compartment decreased with an increase in the chain length of the homologs, as evidenced by the decrease in the k_{12}/k_{21} ratios. Therefore, it may be inferred that the transport of these compounds from the central compartment into the peripheral compartment does not involve a carrier mechanism. Furthermore, since the molecules of IV and its homologs are completely ionized in the systemic body fluids, the solubilities of the compounds in the lipoidal membranes can be considered negligible.

It is generally believed that the ions dissolve in the membrane by interacting with the oppositely charged constituents of the membrane, and if they are transported across the membrane, they do so because the membrane constituent(s) serves as carrier(s). Although the carrier mechanism for the transmembrane transport for IV and its homologs is discounted, as discussed here, it seems possible to explain the transport of these compounds between the central and peripheral compartments by considering the aqueous pore mechanism, especially in view of the magnitudes of the ratios of their distribution rate constants. The diffusion of organic nonelectrolytes through plasma membranes was shown (24, 25) to be governed by their ability to hydrogen bond with membrane components in addition to their lipid solubilities and molecular size. The larger molecular size and greater extent of hydrogen bonding of the permeant molecules decrease their rate of diffusion through the aqueous pores. Since the lining of these aqueous pores is composed of proteins and phospholipids, the diffusion rates of IV and its homologs, with their polar or ionic groups exposed to the aqueous phase of the pores, from one compartment into another may be influenced by their interaction with the ionic or polar portions of the proteins or phospholipids of the pores through electrostatic interaction, hydrogen bonding, and hydrophobic bonding.

In view of these considerations, it seems possible to explain why the volume of the peripheral compartment and the rates of diffusion of V–VII through the aqueous pores are lower than those of IV. It is reasonable to expect that, because of the presence of alkyl groups, neither the charge densities of V–VII nor the hydrogen bonding ability of the hydroxy groups present in their side chains is going to be different from those of IV. Therefore, the lower k_{12}/k_{21} ratios of distribution constants observed for V–VII, as compared to the ratio for IV, are attributed to the presence of alkyl groups. The lower rates of diffusion of the homologs from the central compartment to the peripheral compartment need not be attributed to the molecular size of these molecules, since the homologs of nonelectrolytes, with molecular weights above 140, have increased rates of membrane permeability with an increase in their molecular weights (24).

It is fairly well established that the structure of water within the membrane, including that in the aqueous pore, is much different than the bulk of water, since icebergs are formed by the water molecules present within the membrane in association with the nonpolar portions of phospholipids and/or protein (26, 27). Therefore, if the interactions between the permeant molecules and the protein or phospholipid molecules of the aqueous pores occur through electrostatic forces and/or hydrogen bonding, the nonpolar portions of the permeant molecules and the stationary receptor site(s) of the aqueous pores (proteins and/or phospholipids) may interact through hydrophobic bonding, thus releasing the water molecules associated with the nonpolar portions of these interactants and giving rise to increases in entropy. The association of these reactants then would be stronger, as shown previously in model copolymer system studies (28, 29). This would then result in a decrease in the rate of diffusion of V–VII and, consequently, in a decrease in the volume of the peripheral compartment.

The difference observed between V_2 values of V and VI cannot be ascertained in view of how these values were calculated in the present study; therefore, these values may be considered to be similar. However, the difference between the V_2 values of V and VII appears to be substantial. In view of the mechanism suggested for the transmembrane transport of these organic anions and the role that hydrophobic bonding (between the permeant organic anions and the constituents of the lining of the aqueous membrane pores) plays in reducing the rate of transmembrane transport, the higher V_2 observed for VII (higher even than for V) can be attributed to its branched group, which apparently may not form as strong a hydrophobic bond as does V, since the isopropyl group may not allow close contact of the phenyl group with the hydrophobic portion of the receptor protein or phospholipid of the aqueous pore lining.

As pointed out previously, the V_1 noted for V and VI is somewhat (about 15–20%) lower than that for IV. In view of the fact (discussed in Ref. 30) that, in the presence of a renal tubular inhibitor (DL-tropic acid), the V_1 for IV or VII decreased but that for V or VI remained unchanged, it seems possible to explain the lower V_1 observed for V or VI in terms of the heteroporosity of the membranes. It is known that the membrane pores of a given organ are heteroporous, with their pores ranging in size from 8 to 320 Å; the majority of the pores have a pore diameter of 70–120 Å (31–33). When considering the diameter of the molecule of IV and its homologs, especially when they are hydrated, it seems reasonable to expect that only one molecule of any of these compounds could pass at a time through those pores, which are about 8 Å in diameter. The percentage of small size (8 Å diameter) pores in a membrane of a given organ is apparently not reported in the literature; but if it is assumed that the pores of this size constitute a significant population of the total pores, it is conceivable that molecules showing greater intermolecular interactions with constituents of the pore lining would not be able to occupy the same volume of distribution in a given organ as would similar size molecules exhibiting less intermolecular interactions. Therefore, it is believed that V and VI probably show greater interactions with the aqueous pore lining through hydrophobic bonding than do IV and VII. Consequently, the V_1 for V or VI can be expected to be smaller than that for IV or VII.

Comparison of Effect of Carbonyl Group of I with Effect of Alcoholic Hydroxy Group of IV—The striking effect of the carbonyl group of I, as compared to that of the hydroxy group of IV, becomes apparent from the fact that the disappearance of I from the rat blood is describable by a three-compartment open model whereas that of IV is describable by a two-compartment open model, even though the biological half-lives of the compounds determined from their respective predominantly elimination phases are very similar. One possible explanation for this difference follows.

The rate of diffusion of the molecules of IV through the aqueous pores of tissue membranes, which constitute the peripheral compartment, can be expected to be different due to the differences in the chemical composition of the lining of the pores of different tissues. Apparently, however, such differences in the rates of diffusion do not exist so as to distinguish the various tissues of the peripheral compartment into more than one peripheral compartment. However, the differences in the rates of diffusion of the molecules of I through the aqueous pores of the peripheral compartment tissues may vary widely because they have less ability to hydrogen bond, as compared to those of IV; therefore, the various tissues of the peripheral compartment become kinetically distinguishable as two peripheral compartments. The reason for expect-

ing the molecules of I to show these differences is that the chemical composition of proteins and phospholipids of the membranes of all tissue organs (34) and, therefore, of their aqueous pore lining is not identical. It is conceivable, therefore, that the molecules of I, which have one less potential or a weaker site for hydrogen bonding than the molecules of IV, could show relatively greater hydrogen bonding with the constituents of the lining of pores of the one tissue organ than with the constituents of the lining of pores of other tissue organs of the peripheral compartments.

On this basis, those organs whose aqueous pores exhibit stronger hydrogen bonding with the molecules of I would constitute the peripheral compartment that would have a smaller apparent volume of distribution; organs whose aqueous pores exhibit weaker hydrogen bonding with the molecules of I would constitute the peripheral compartment that would have a larger apparent volume of distribution. The former peripheral compartment may be designated as peripheral compartment 1 with volume V_2, and the latter peripheral compartment may be designated as peripheral compartment 2 with volume V_3. As stated previously, if the differences in the extent of hydrogen bonding of IV with the constituents of the aqueous pores of these peripheral compartments are not sufficiently large, these peripheral compartments would then be kinetically describable as a single peripheral compartment for IV.

It is observed that the volume of the central compartment for I is larger than that for IV, and the volume of its peripheral compartments ($V_2 + V_3$) is smaller than that for the molecules of IV. In connection with the transport of these molecules through the aqueous pores, it seems possible that I is able to equilibrate into part of the compartment that serves as a peripheral compartment of IV somewhat more readily than does IV, thus resulting in a larger volume of the central compartment. Also, since the volume of the peripheral compartment is in no sense an absolute value of the actual volume but rather a mathematically manipulated value, the lower volume of the peripheral compartments ($V_2 + V_3$) for I may possibly be explained on the basis that I probably interacts with the biopolymers of the intracellular fluids of the tissue cells of the peripheral compartments, thereby restricting its diffusion into only a limited region of the tissues of the peripheral compartments. The reason for speculating about the interaction of I with the intracellular biopolymers is based on the observation that I is bound to plasma protein to the extent of 13–15% while IV shows no binding to plasma proteins (9). The binding of drugs to tissue proteins, such as nucleic acids and DNA, is reported in the literature (34).

Effect of Substitution of Alkyl Groups in the *para*-Position of I—Since the blood level data for I can be described by a three-compartment open model, the revelation that the blood level data for II and III can be described by a one-compartment open model demonstrated a dramatic effect of alkyl groups on the distribution of these compounds in rats.

Among IV–VII, although the effect of *para*-alkyl groups is not seen in terms of reducing the two-compartment open model for IV to a one-compartment open model for V–VII, the dramatic effect of the alkyl groups in terms of a substantial decrease in the volume of the peripheral compartment is observed (Table V). However, as pointed out later, blood level data for D-(−)-*p*-*n*-propylmandelic acid are indeed describable by a one-compartment open model (13), thereby demonstrating the effect of the propyl group in reducing the volume of distribution of the peripheral compartment to zero, as observed for II and III. Clearly, for the *para*-alkylated homologs of I and IV, the apparent effect of the alkyl groups is to diminish the volume of the peripheral compartment, but the disappearance of a kinetically describable peripheral compartment remains a function of the size of the peripheral compartment for the parent compounds.

Accordingly, if the volume of the peripheral compartment is relatively large for the parent compound, the peripheral compartment may not become kinetically indistinguishable for the short alkyl chain *para*-alkylated homologs as seen for V–VII. However, if the volume of the peripheral compartment is relatively small for the parent compound, it may become kinetically indistinguishable, even for the short alkyl chain *para*-alkylated homologs as noted for II and III. As explained in the case of the homologs of IV, the effect of alkyl groups in restricting the distribution of the compound to the central compartment is a consequence of the decreased rate of diffusion of the compounds due to the strengthened interaction (through hydrophobic bonding) of the permeant molecules with the proteins and/or phospholipids of the lining of the aqueous pores of the tissue organs.

The volume of the central compartment noted for II is slightly greater than that for I. This slightly higher volume may be due to incorporation of that volume of the peripheral compartment that is not kinetically distinguishable because of its highly reduced size. It is believed that, since the rate of diffusion of II from the central compartment into the peripheral compartment is considerably reduced, the amount of the compound distributed into the peripheral compartment is relatively small, and the system cannot be kinetically described either as a two-compartment open model or a three-compartment open model.

If the pattern observed for I and its homologs with respect to their volumes of the central compartment is considered, the volume of the central compartment for III would be anticipated at 225–250 ml, which is the range of the volume of the central compartment observed for I and II. However, the volume of the central compartment noted for III is only about 125 ml. A study is under consideration to determine the possible reason for this finding.

Comparison of Effect of Branched Alkyl Group (Isopropyl) against Straight Chain Alkyl Group (Propyl)—The kinetics of disappearance of D-(−)-*p*-*n*-propylmandelic acid (VIII), which also possesses the "ideal properties," from the blood has been studied in rats (13). The pharmacokinetics of this compound are describable by a one-compartment open model, and its apparent volume of distribution is 220 ml. The dramatic effect of hydrophobic bonding in reducing the rate of transmembrane transport through aqueous membrane pores is readily seen with VIII, as with II and III. Clearly, the propyl group of this compound is able to form a substantially stronger hydrophobic bond with constituents of the membrane pores, thus restricting the compound mainly in the central compartment of distribution.

Although the total volume of distribution (one-compartment open model) observed for VIII is 20% larger than that observed for the central compartment (two-compartment open model) of VII, the volume of distribution of the central compartment for VIII may not be larger than that observed for VII or for that matter for IV–VI. It is believed that, since the rate of diffusion of VIII from the central compartment into the peripheral compartment is considerably reduced, the amount of the compound distributed into the peripheral compartment is relatively small and the system cannot be kinetically described as a two-compartment open model.

The influence of the geometrical factor relating to the extent of surface contact area between the permeating molecule and the membrane was illustrated by Diamond and Wright (25). They showed that the branched nonelectrolytes such as isobutyramide and isovaleramide exhibited less permeability in several epithelia than did their corresponding straight chain isomers butyramide and valeramide. This observation is considered to be very striking because the partition coefficients of the straight chain isomers are noted to be lower than those of the branched isomers and, in water, the diffusion coefficients of butyramide and isobutyramide are similar (35). The possible explanation for this observation is that the area of close contact with surrounding molecules decreases with increased branching, so the van der Waals forces between the permeating molecule and the membrane are weakened. This explanation suggests that the selectivity is determined by the types of interactions in the membrane interior which, in the case of these molecules, implies that the rate-limiting step is diffusion through the membrane and not transition from aqueous solution to the membrane phase (35).

In the case of the isomers of organic nonelectrolytes, the isomers containing branched alkyl groups showed less permeability into the epithelial membrane than did the corresponding isomers containing straight chain alkyl groups (25); in the case of organic electrolytes of the present study, the isomer containing a branched alkyl group (VII) showed a greater rate of permeability through the aqueous pores of membranes than did the corresponding isomer containing a straight chain alkyl group (VIII). If, indeed, the major pathway for the transfer of VII and VIII anions from the central body compartment into the peripheral body compartment was through the membrane (possibly *via* a "carrier" mechanism), the pharmacokinetics of VIII would probably have been describable according to the two-compartment open model, with V_2 greater than that noted for VII and possibly IV. Therefore, this observation is supportive of the aqueous pore mechanism suggested for transmembrane transport of I, IV, and some of its *para*-alkylated

homologs between central and peripheral body compartments.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1974, from the *Pharmaceutics Division, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI 48202*

Accepted for publication February 25, 1975.

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, November 1974.

Abstracted in part from a dissertation submitted by Y. M. Amin to the Graduate Division, Wayne State University, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by the Faculty Research Grant-in-Aid Program of Wayne State University.

* Recipient of a Wayne State University Graduate Fellowship, 1973–1974.

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