Effect of Complex Formation on Drug Absorption XI: Complexation of Prednisone and Prednisolone with Dialkylpropionamides and Its Effect on Prednisone Transfer through an Artificial Lipoid Barrier

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Abstract □ Complex formation of *N,N*-dimethyl-, *N,N*-diethyl-, *N,N*-di-*n*-propyl-, and *N,N*-di-*n*-butylpropionamide with prednisone and prednisolone was studied by solubility and partition methods. The steroid–propionamide interaction is greater in isopropyl myristate than in water. The size of the *N*-substituent has no apparent effect on the interaction in isopropyl myristate. The apparent isopropyl myristate/water partition coefficient of the steroids is decreased by *N,N*-dimethyl- and increased by *N,N*-diethyl-, *N,N*-di-*n*-propyl-, and *N,N*-di-*n*-butylpropionamide. The propylamide increased the rate of transfer of prednisone and prednisolone from an aqueous solution through an artificial lipoid barrier. A mathematical model based on formation of a prednisone–*N,N*-di-*n*-propylpropionamide complex in the barrier was developed, and the apparent diffusion coefficients for prednisone and the prednisone–amide complex in the barrier phase were determined. These studies provide the physicochemical basis for investigations of the effect of *N,N*-dialkylpropionamides on the intestinal absorption of prednisone and prednisolone.

Keyphrases □ Drug absorption, prednisone and prednisolone—effect of steroid–dialkylpropionamide complex formation on transfer through artificial lipoid barrier □ Complex formation, prednisone/prednisolone–dialkylpropionamide—effect on transfer through artificial lipoid barrier □ Prednisone–dialkylpropionamide complex formation—effect on transfer through artificial lipoid barrier □ Prednisolone–dialkylpropionamide complex formation—effect on transfer through artificial lipoid barrier □ Dialkylpropionamides—effect of complex formation on steroid drug absorption, transfer through artificial lipoid barrier

There has been considerable interest recently in the possible use of complex formation as a means of increasing the absorption rate of drugs (*e.g.*, 1–4). In theory, the idea of enhancing the absorption of poorly absorbed drugs by forming well-absorbed complexes is very attractive in that drug absorption and availability problems might be solved without chemical modification of the drug or alteration of biologic barriers. The idea is based in part on the well-known fact that many complexes are much more lipophilic than either of the interacting constituents. In practice, however, complex formation in aqueous media (such as the GI fluids) has not yet proven to be a particularly useful approach to the solution of drug absorption problems.

While previous investigations focused largely on complex formation in the aqueous solution bathing the biologic barrier, the influence on drug absorption of complexing agents that complex with drugs primarily in the biologic barrier has not been explored formally. Biologic membranes have the characteristics of lipoid barriers (5). It is likely, therefore, that complexes which form in organic solvents may also form in the lipoid environment of biologic membranes and thereby alter drug absorption rates. For this reason, the authors studied the interaction of a homologous series of dialkylpropionamides with prednisone and prednisolone in water and in an organic solvent, preliminary to an investigation of the influence of such interaction on the absorption of the steroids from the small intestine of the rat. This report deals with the characteristics of the steroid–dialkylpropionamide complexes and with the effect of one of the propionamides on the transfer of the steroids through an artificial lipoid barrier.

EXPERIMENTAL

Materials—Prednisone USP[1] and prednisolone USP[2] were used. The propionamides[3], *N,N*-dimethyl-, *N,N*-diethyl-, *N,N*-di-*n*-propyl-, and *N,N*-di-*n*-butylpropionamide (methyl-, ethyl-, propyl-, and butyl-amide, respectively), were distilled at reduced pressure before use. Isopropyl myristate[4], sodium salicylate[5], and other chemicals of reagent grade were used as received from the supplier.

Assay Procedures—Prednisone and prednisolone concentrations in the solubility and equilibrium partition experiments were determined by the USP colorimetric procedure (6). Prednisone and prednisolone concentrations in the lipoid barrier studies were determined by the colorimetric method of Porter and Silber (7). Salicylate concentrations were determined by the colorimetric procedure of Trinder (8). Ethyl-amide concentrations were determined by GC using a gas chromatograph[6] with a flame-ionization detector. A stainless steel column, 1.83 m. (6 ft.) long and 0.32 cm. (0.125 in.) o.d., packed with 15% didecyl phthalate on Chromosorb W (80/100 mesh) was used[7]. The column temperature was 125°, and the injection port and detector temperatures were approximately 180°. The flow rates of helium (carrier gas), hydrogen, and air were approximately 30, 30, and 350 ml./min., respectively. Ethyl-amide-containing samples were diluted with acetone; dimethylacetamide was added as an internal standard, and 1-μl. aliquots were injected into the chromatograph set at a full scale sensitivity of 1, 2, or 5×10^{-10} amp. Under these conditions, a plot of the area of the amide peak divided by the area of the internal standard peak as a function of the amide concentration was linear and passed through the origin. The retention times of dimethylacetamide and the ethyl-amide were 3.6 and 8.5 min., respectively, and the maximum currents at their peaks were 1.75×10^{-10} and 2.35×10^{-10} amp., respectively, following injection of 1 μl. of a solution containing 0.5% of each amide.

Solubility Studies—Fifteen milligrams of steroid was placed in each of a series of glass vials. Five milliliters of a solution of the amide in water or isopropyl myristate was added to each vial, and the vials were sealed with polyethylene stoppers which were secured with a rubber band placed around the long axis of the vial. The vials were rotated for 24 hr. in a 25.0 ± 0.1° water bath and then placed upright for several hours to allow suspended particles to settle. A sample of the supernatant was removed with a pipet and filtered through a Millipore filter (type HA, 0.45 μ), and the steroid concentration was determined. No change in steroid concentration was observed when a solution of the steroid was passed through the

[1] Parke, Davis & Co., Detroit, Mich.
[2] The Upjohn Co., Kalamazoo, Mich.
[3] Eastman Organic Chemicals, Rochester, N. Y.
[4] Emery Industries, Inc., Cincinnati, Ohio.
[5] Fisher Scientific Co., Fair Lawn, N. J.
[6] Perkin-Elmer, model 811.
[7] The column was prepared by Perkin-Elmer.

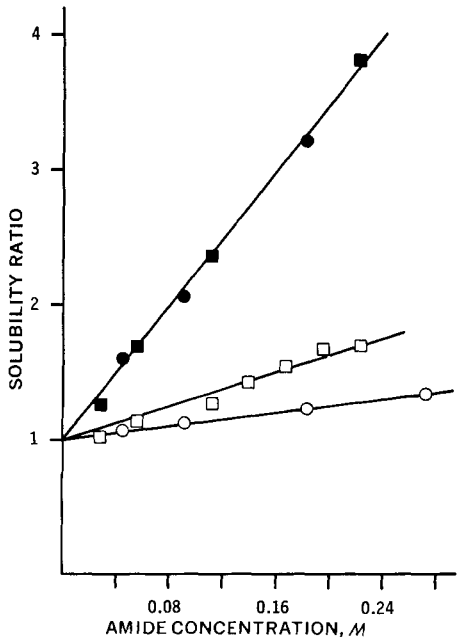

Figure 1—*Apparent solubility of prednisone as a function of amide concentration in isopropyl myristate and water. Key: ■, propyl-amide in isopropyl myristate; ●, methyl-amide in isopropyl myristate; □, propyl-amide in water; and ○, methyl-amide in water. Apparent solubility is expressed as a ratio relative to the solubility of prednisone in the pure solvent.*

filter. The vials were resealed and rotated for an additional 15 hr., and the steroid concentration again was determined to ensure that equilibrium had been attained.

Equilibrium Partition Studies—To determine the apparent partition coefficient of the steroids in the presence of an amide, the amide was weighed into a series of glass vials and 2 ml. of isopropyl myristate and 2 ml. of an aqueous solution of steroid were added to each vial. The vials were sealed and agitated as in the solubility studies for several hours, and the steroid concentration in the isopropyl myristate phase was determined. The steroid concentration in the aqueous phase was determined by difference. A correction was made for phase volume changes due to the addition of the amide. The partition coefficient of the ethyl-amide was determined in a similar manner at 25° by adding the amide to the described system and measuring its concentration in each phase.

Lipoid Barrier Studies—Equilibrium dialysis cells (Plexiglas) with two 10-ml. compartments separated by a Millipore filter saturated with isopropyl myristate were used. The cell was round with an internal diameter of 4 cm. and accommodated a 4.7-cm. diameter filter. White, plain, polyethylene filters (type OH), with 1.5 ± 0.5-μ pore size, a thickness of 127 ± 13 μ, and a void volume[8] of 70%, were used (9). Ten milliliters of the isopropyl myristate phase and 40 ml. of the aqueous phase were equilibrated and separated, the drug was dissolved in a portion of the aqueous phase, and the filter was soaked in the isopropyl myristate phase.

The initial concentrations of steroid and salicylic acid were 3.0×10^{-4} and 1.2×10^{-2} *M*, respectively. When propyl-amide was present, it was added to the system prior to equilibration, and the volumes of lipoid and buffer solution were reduced to compensate for the amide volume. The filters were weighed dry and after being soaked in the isopropyl myristate phase and blotted with filter paper. The membranes were weighed again at the end of each experiment after they had air dried for several days. The cells were assembled with the membrane in place, and 10 ml. of the aqueous sink phase, without drug, was placed in one compartment and the membrane was examined for leaks. Ten milliliters of drug solution was then placed in the source compartment, the sampling ports were sealed, and the cells were rotated at 100 r.p.m. for the salicylate study and 40 r.p.m. for the prednisone study in a 25° water bath.

[8] This was taken into consideration when calculating the effective surface area of the barrier. See Eq. A1 and Footnote 10.

Table I—Solubility[a] of Prednisone and Prednisolone in Water and Isopropyl Myristate, and the Partition Coefficient of the Steroids in Isopropyl Myristate/Water at 25°[c]

	Prednisone	Prednisolone
Solubility in water	3.71 (0.18)	6.74 (0.13)
Solubility in isopropyl myristate	1.40[b]	5.62 (0.05)
Isopropyl myristate/water partition coefficient	0.458 (0.033)	0.398 (0.039)

[a] Solubility in $M \times 10^4$; the numbers in parentheses are standard deviations of the means of four to five determinations except where indicated otherwise. [b] Only two determinations were made.

In the salicylate study, the sink compartment was maintained at pH 7 and the source compartment pH was varied from 1 to 7 with the appropriate citrate or phosphate buffer or 0.1 *N* HCl. In the steroid studies, the solutions in the source and sink compartments were not buffered. The partition coefficient of prednisone between each isopropyl myristate and aqueous phase was determined by rotating 2 ml. of the source phase and 2 ml. of the isopropyl myristate filter phase in a glass vial for several hours and determining the prednisone concentration in each phase.

Viscosity Determinations—Viscosity was determined at 25 ± 0.05° with a viscometer (Ostwald). The viscometer was standardized with distilled water, and the viscosity of isopropyl myristate and of solutions of propyl-amide in isopropyl myristate was calculated by:

$$\eta = r \cdot \eta_{H_2O}^{25^\circ} \cdot \left(\frac{t}{t_{H_2O}}\right) \qquad \text{(Eq. 1)}$$

where η is the unknown viscosity; $\eta_{H_2O}^{25^\circ}$ is the viscosity of water at 25°; t and t_{H_2O} are the times required for the movement of equal volumes of the unknown and water, respectively, through the capillary; and r is the ratio of the density of isopropyl myristate to the density of water at 25°. Efflux rates were sufficiently low ($t > 10$ min.) to assure laminar flow.

RESULTS AND DISCUSSION

Solubility Studies—The solubilities of prednisone and prednisolone in water and isopropyl myristate are presented in Table I. Methyl- and propyl-amide increased the apparent solubility of the steroids in water and isopropyl myristate (Figs. 1 and 2), indicating

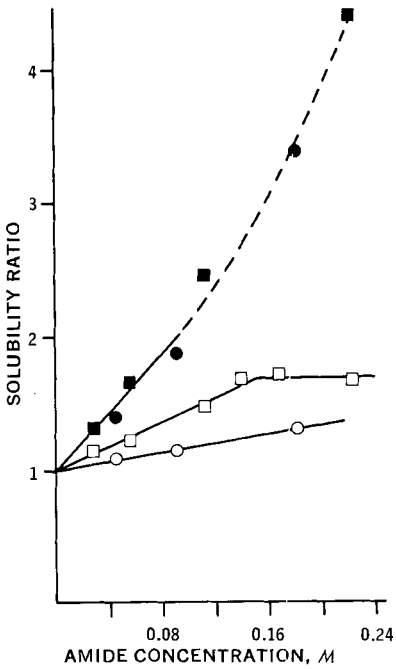

Figure 2—*Apparent solubility of prednisolone as a function of amide concentration in isopropyl myristate and water. Symbols are the same as in Fig. 1.*

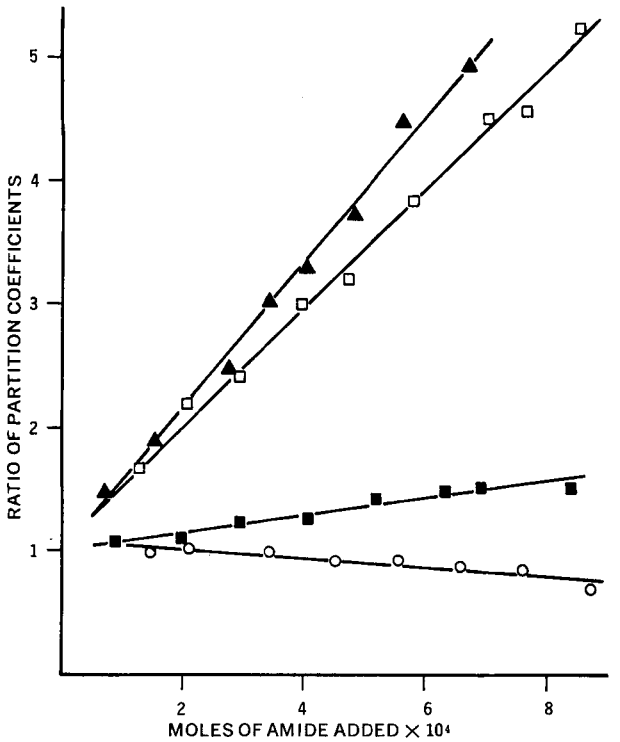

Figure 3—*Apparent partition coefficient of prednisone between 2 ml. isopropyl myristate and 2 ml. water as a function of the amount of amide added to the system. Key: ○, methyl-amide; ■, ethyl-amide; □, propyl-amide; and ▲, butyl-amide.*

that the amides and steroids associate to form one or more types of complexes (10). By assuming that one molecule of steroid complexed with one molecule of amide, the slopes of the plots in Figs. 1 and 2 yield an apparent association constant of 12.5 M^{-1} for a complex of prednisone or prednisolone and either methyl- or propyl-amide in isopropyl myristate. In water, the apparent 1:1 association constant is about 1.2 and 3.1 M^{-1} for the prednisone–methyl-amide and prednisone–propyl-amide complexes, respectively, and 1.7 and 4.5 M^{-1} for the prednisolone–methyl-amide and prednisolone–propyl-amide complexes, respectively. These results are consistent with the findings of Shami (11), who reported that the size of the alkyl substituent on the amide nitrogen did not affect the magnitude of the association constant of a *p*-nitrophenol–alkylricinoleamide complex in organic solvents.

The increase in the apparent association constant in aqueous solvents with an increase in the size of the substituent on the amide nitrogen was reported by Kostenbauder and Higuchi (12) and was interpreted as a " 'squeezing out' effect of water on the relatively hydrophobic complexing agent." The discontinuity in the solubility diagram for prednisolone in aqueous solutions of propyl-amide (Fig. 2) indicates that the complex precipitates at propyl-amide concentrations above 0.15 M (10). The stoichiometry of the steroid–amide complex was not determined; deviation of the solubility plots from linearity at high amide concentrations indicates that complexes having more than one amide molecule may be formed (10).

Table II—Partition Coefficient of Ethyl-Amide at Several Concentrations

Moles of Ethyl-Amide Added[a] × 10^4	Partition Coefficient
1.12	0.415
2.49	0.397
3.55	0.379
5.36	0.393
6.39	0.404
Mean	0.398

[a] Moles of ethyl-amide added to a system of 4.00 ml. each of isopropyl myristate and water at 25°.

Table III—Uptake and Loss of Lipoid by the Barrier Matrix[a] at Several Concentrations of Propyl-Amide in the Isopropyl Myristate Phase

Percent Propyl-Amide in Isopropyl Myristate	Filter Weight Alone, mg.	Uptake[b], mg. of Lipoid Phase/ mg. of Filter	Loss[c], Percent of Lipoid Phase
0	73.3	1.49	20.6
	72.4	1.49	19.7
	72.9	1.50	20.9
1	73.6	1.51	17.9
	74.9	1.50	18.2
2	76.5	1.51	22.9
	75.4	1.52	20.1
4	73.0	1.50	24.7
	75.3	1.50	28.8
	73.4	1.49	24.8
	73.7	1.49	25.3

[a] Millipore filter, type OH. [b] The filter was soaked in isopropyl myristate containing 0–4% propyl-amide, and blotted with dry filter paper. [c] At the end of the experiment (*i.e.*, after 6–30 hr. of exposure to the aqueous phase).

Partition Studies—The apparent partition coefficients of prednisone and prednisolone between isopropyl myristate and water are listed in Table I. The apparent partition coefficients of the steroids were decreased somewhat by methyl-amide and increased by ethyl-, propyl-, and butyl-amides (Figs. 3 and 4). The apparent partition coefficients of the steroids in the presence of a given molar amount of amide in the system increased as the size of the nitrogen substituents of the amide increased from ethyl to *n*-butyl. The apparent distribution of the steroids in the partitioning system involves at least five simultaneous equilibria (13). These are the equilibria between the complex and its constituents in isopropyl myristate and water, and the respective distribution of the amide, steroid, and complex between isopropyl myristate and water. There was no apparent self-association of the amides in either isopropyl myristate

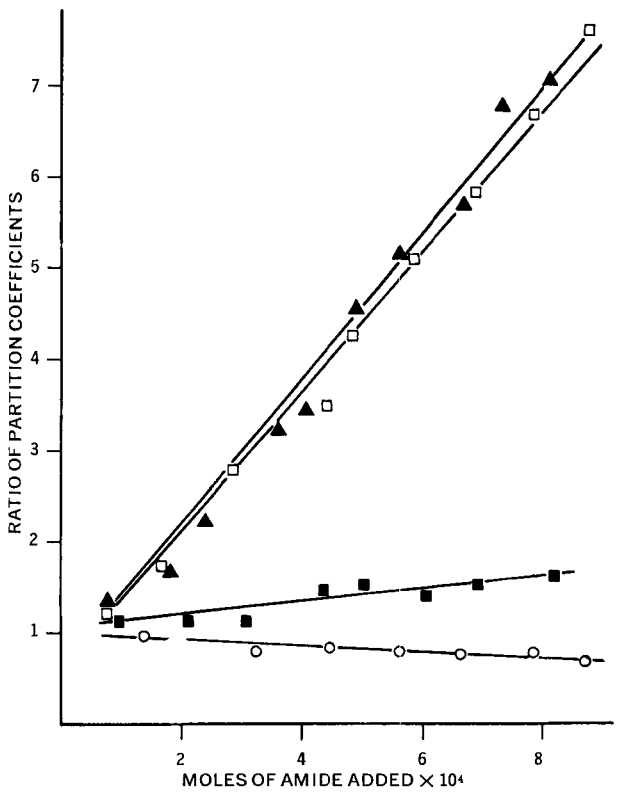

Figure 4—*Apparent partition coefficient of prednisolone between isopropyl myristate and water as a function of the amount of amide added to the system. Symbols are the same as in Fig. 3.*

Table IV—Effect of Propyl-Amide on the Viscosity of Isopropyl Myristate at 25°

Percent Propyl-Amide	Viscosity, cps.
0	4.69
1	4.68
2	4.66
4	4.57

or water as indicated by the concentration independence of the partition coefficient of ethyl-amide (Table II). Using a cryoscopic method, Chaplin and Hunter (14) also found that *N,N*-disubstituted amides do not associate in benzene.

Lipoid Barrier Studies—The dry weights of each Millipore filter used to prepare the lipoid barriers were nearly the same, and the weight of isopropyl myristate taken up by the filters was reproducible and not affected by added propyl-amide (Table III). Since the densities of isopropyl myristate and propyl-amide are similar (0.85 and 0.88 g./ml., respectively), it may be concluded that the thickness of the barrier is not affected by incorporation of the amide. The viscosity of isopropyl myristate was also not affected significantly by the amide (Table IV), indicating that any change in permeability of the barrier was not due to altered viscosity and its effect on the diffusion of drugs through the barrier. About 20% of the lipoid phase was lost during the experiment. This loss was due almost entirely to the "squeezing out" of lipoid from the edge of the barrier when the latter was compressed between the rims of the diffusion cell. There was no time-dependent change in the permeability characteristics of these membranes in the kinetic studies, and there was no relationship between the loss of lipoid phase and the duration of the experiment. This is consistent with the direct observation that almost all the weight loss of the lipoid barrier occurs while mounting the barrier in the cell.

The lipoid characteristics of the barrier were investigated by studying the transfer of salicylic acid from aqueous buffer solutions of pH 1.29–7.0 to an aqueous sink of pH 7.0 (Fig. 5). The transfer of salicylic acid across the barrier followed apparent first-order kinetics and was proportional to the concentration gradient of nonionized salicylic acid both in the absence and presence of propyl-amide (Fig. 6), indicating that the barrier has ideal lipoid characteristics with and without propyl-amide in the sense that it can maintain a pH gradient and is impermeable to lipoid-insoluble ionized species. However, the apparent rate constant for transfer of salicylic acid increased in the presence of propyl-amide. This was probably due to formation of a salicylic acid–amide complex in the barrier, since

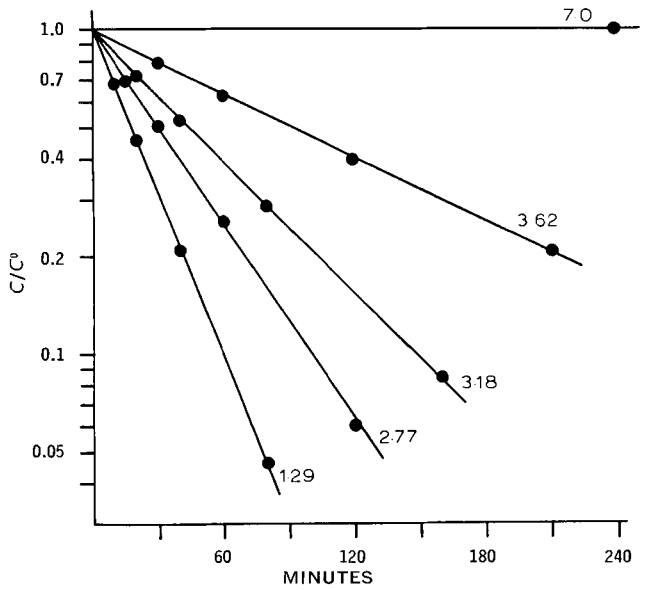

Figure 5—*Decrease of salicylic acid concentration in the source compartment as a function of time. The pH of the receiving compartment was 7; the pH of the source compartment is shown next to the corresponding plot.*

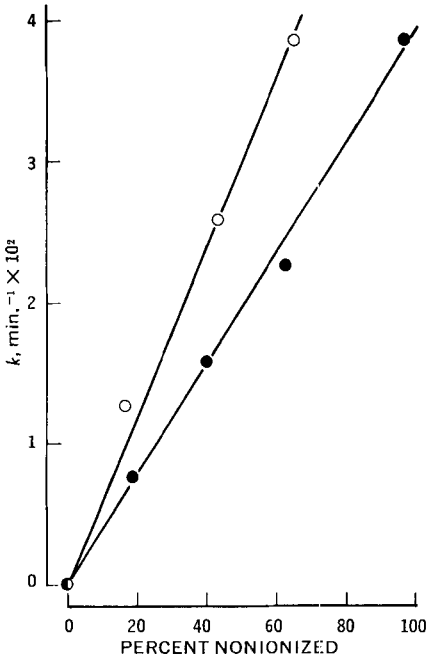

Figure 6—*Apparent first-order rate constant for salicylic acid transfer through the lipoid barrier as a function of the percent of salicylic acid in nonionized form in the source compartment. Key: ●, no amide added; and ○, with 1% propyl-amide in the barrier phase.*

phenols and disubstituted amides are known to interact strongly in organic solvents (15).

Prednisone transfer across the lipoid barrier followed apparent first-order kinetics, with the rate of transfer being proportional to the concentration gradient of prednisone across the barrier (Fig. 7). The apparent first-order rate constant was independent of stirring speed in the range tested (Table V) and increased as the concentration of propyl-amide in the barrier increased. Similar results were obtained with prednisolone. A plot of the apparent first-order rate constant for prednisone transfer as a function of the apparent partition coefficient of prednisone between the barrier phase and water is

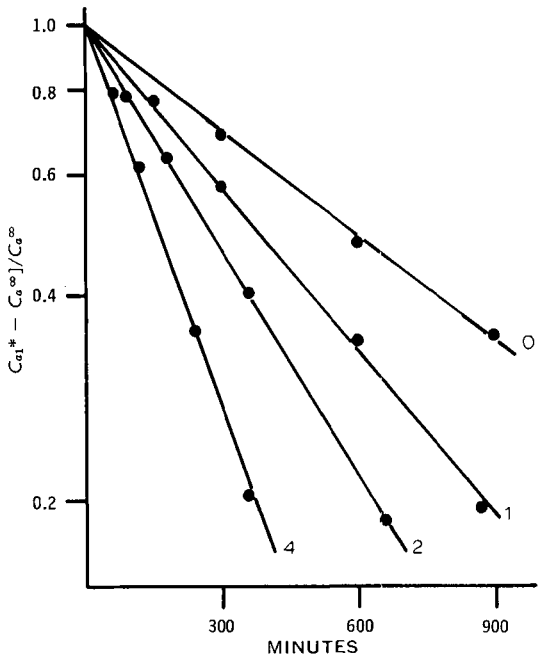

Figure 7—*Effect of propyl-amide on the transfer of prednisone through a lipoid barrier. The graph shows the decrease of prednisone concentration in the source compartment as a function of time with 0, 1, 2, and 4% amide in the barrier phase.*

Table V—Influence of Stirring Rate on the Apparent First-Order Rate Constant ($k_{app.}$) for Prednisone Transfer through the Lipoid Barrier Containing 4% Propyl-Amide

Stirring Rate, r.p.m.	$k_{app.}$, min.$^{-1}$ × 10^3
40	4.25
	4.12
100	4.33
	4.07

linear and has a positive intercept significantly ($p < 0.05$) different from zero (Fig. 8). By assuming that: (*a*) the resistance to prednisone transfer across the barrier is within the barrier, and (*b*) the complex-forming reaction is at equilibrium throughout the system, it can be shown that the positive intercept in Fig. 8 is a result of the diffusion coefficient of prednisone being greater than that of the prednisone–propyl-amide complex (Eq. A13). From the slope and intercept of the plot in Fig. 8 and other necessary parameters specified in the *Appendix*, the diffusion coefficients of prednisone and the prednisone–amide complex in the barrier phase were calculated to be 2.98 × 10^{-7} and 2.27 × 10^{-7} cm.^{2} sec.$^{-1}$, respectively. The diffusion coefficient of prednisone determined in experiments without amide was 3.09 × 10^{-7} cm.^{2} sec.$^{-1}$, in good agreement with the value calculated from the results of the experiments with the complexing agent. The ratio of the two diffusion coefficients is 1.3, which is reasonable considering the molecular weight of 358 for prednisone and 515 for the prednisone–propyl-amide complex (assuming 1:1 stoichiometry)[9].

The positive intercept in Fig. 8 is consistent with the concept of simultaneous diffusion of both free and complexed prednisone

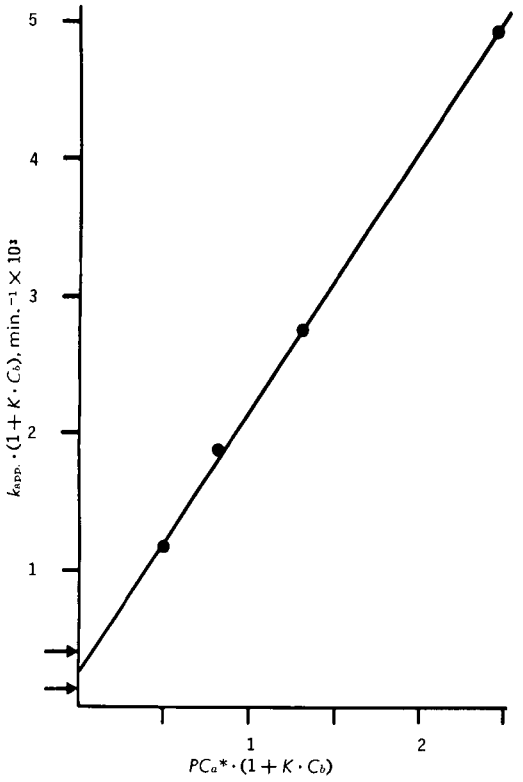

Figure 8—*Apparent first-order rate constant* ($k_{app.}$) *for prednisone transfer through the lipoid barrier as a function of the apparent barrier phase/water partition coefficient of prednisone* (PC_a^*). *Arrows indicate the 95% confidence limits of the intercept on the ordinate. Both* $k_{app.}$ *and* PC_a^* *have been multiplied by* $(1 + K \cdot C_b)$ *to compensate for complex formation in the aqueous phase (see Eq. A12).*

across the barrier. Thus, it is suggested that the enhanced transfer of the steroid across the lipoid barrier in the presence of amide is due to complex formation between these species within the barrier (at the highest propyl-amide concentration used in this study, 75% of the steroid in the barrier was complexed with the amide). Similar complex formation might be anticipated in biologic barriers in view of their lipoid characteristics. An exploration of this possibility and of its consequences with respect to the intestinal absorption of prednisone and prednisolone is the subject of the next paper in this series (16).

APPENDIX

The stationary-state rate of transfer, dM_a/dt in amount per time, of a substance, *A*, from a well-stirred aqueous source solution through a lipoid barrier to a well-stirred aqueous receiving solution may be described by the expression:

$$\frac{dM_a}{dt} = \frac{-D_a \cdot S}{L}(C_{a_1}' - C_{a_2}') \qquad \text{(Eq. A1)}$$

where D_a is the diffusion coefficient of *A* in the barrier; *S* and *L* are the effective surface area[10] and the effective thickness[11] of the barrier, respectively; and C_{a_1}' and C_{a_2}' are the concentrations of *A* at the source and receiving surfaces in the barrier. It is assumed that no significant resistance to transfer of *A* from the source to the receiving solution occurs in aqueous diffusion layers and that *A* partitions essentially instantaneously between the barrier surfaces and aqueous phases. If the volumes of the source and receiving solutions are equal and the concentration of *A* in the membrane is related to the concentration of *A* in the aqueous source (C_{a_1}) and receiving (C_{a_2}) solutions through a partition coefficient, PC_a, then $C_{a_1}' = PC_a \cdot C_{a_1}$ and $C_{a_2}' = PC_a \cdot C_{a_2}$.

Equation A1 can be integrated:

$$\ln\left[\frac{C_{a_1} - C_{a_1}^{\infty}}{C_{a_1}^{\infty}}\right] = -2 \cdot k_a \cdot PC_a \cdot t \qquad \text{(Eq. A2)}$$

where $C_{a_1}^{\infty}$ is the concentration of *A* in the source solution at time *t* equal to infinity, and k_a is composed of several constants:

$$k_a = \frac{D_a \cdot S}{L \cdot V} \qquad \text{(Eq. A3)}$$

where *V* is the volume of the source solution.

When a complexing agent, *B*, is present in such a system at the same concentration in the source and receiving solutions, the following differential equation describes the transfer of *A* from the source solution:

$$\frac{dM_a^*}{dt} = -\frac{D_a \cdot S}{L}(C_{a_1}' - C_{a_2}') - \frac{D_e \cdot S}{L}(C_{e_1}' - C_{e_2}') \qquad \text{(Eq. A4)}$$

where D_e is the diffusion coefficient of the complex *E*; C_{e_1}' and C_{e_2}' are the concentrations of the complex at the source and receiving surfaces in the barrier; and DM_a^*/dt is the rate at which *A* leaves the source compartment in both the free and complexed form. It is assumed that the complex-forming reaction is at equilibrium, that only one type of complex is formed with a 1:1 stoichiometry, and that the concentration of *E* is small compared to the concentration of *B*. From the association constant of the complex in the aqueous and barrier phases, *K* and *K'*, respectively, the following relationships hold:

$$C_{e_1}' = K' \cdot C_{a_1}' \cdot C_b' \qquad \text{(Eq. A5)}$$

$$C_{e_2}' = K' \cdot C_{a_2}' \cdot C_b' \qquad \text{(Eq. A6)}$$

$$C_b' = PC_b \cdot C_b \qquad \text{(Eq. A7)}$$

$$C_{a_1}^* = M_a^*/V = C_{a_1} \cdot (1 + K \cdot C_b) \qquad \text{(Eq. A8)}$$

where C_b and C_b' are the concentrations of *B* in the aqueous and barrier phases, respectively; and PC_b is the barrier/aqueous partition

[9] It is assumed that channels through the barrier matrix are straight and perpendicular to the barrier surface. If the tortuosity is greater than unity, the absolute values for the diffusion coefficients would be somewhat higher but the ratio would be the same.

[10] Effective surface area = surface area × porosity. The porosity of the lipoid barrier used in this study is 0.7.

[11] Effective thickness = thickness × tortuosity.

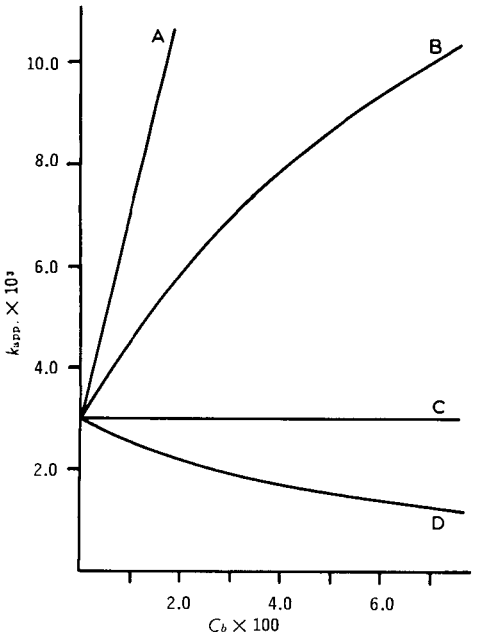

Figure 9—*Effect of complexing agent on the apparent rate constant* ($k_{app.}$ *in* $min.^{-1}$) *for transfer of a substance across a lipoid barrier according to the kinetics presented in the* Appendix. $k_{app.}$, *calculated from Eq. A10, is plotted as a function of the molar concentration of complexing agent* (C_b) *in the aqueous phase.* PC_a *is 1,* PC_b *is 10,* k_a *is 0.0015* $min.^{-1}$, *and* k_e *is 0.0010* $min.^{-1}$ *for all four curves. The association constants in the aqueous and barrier phases* (K *and* K′ *in* M^{-1}) *are, respectively, 0 and 20 for curve* A, *10 and 10 for curve* B, *20 and 3 for curve* C, *and 20 and 0 for curve* D.

coefficient of B. Substituting the relationships in Eqs. A5–A8 into Eq. A4 and integrating give the following equation:

$$\ln\left[\frac{C_{a_1}^* - C_a^\infty}{C_a^\infty}\right] = -k_{app.} \cdot t \qquad \text{(Eq. A9)}$$

where C_a^∞ is the concentration of free and complexed A in the source and receiving solutions at time equal infinity, and $k_{app.}$ is an apparent first-order rate constant composed of a number of other constants:

$$k_{app.} = 2PC_a \cdot \frac{k_a + k_e \cdot K' \cdot PC_b \cdot C_b}{1 + K \cdot C_b} \qquad \text{(Eq. A10)}$$

where k_e is identical to k_a except that the diffusion coefficient is that of the complex (Eq. A3). Equation A9 predicts that in the presence of a constant concentration of complexing agent B, a substance A will cross the barrier by an overall first-order kinetic process as it does in the absence of complexing agent. Equation A10 predicts that the complexing agent can either increase, decrease, or have no effect on the apparent permeability of the barrier (Fig. 9). Except when $K = 0$, $k_{app.}$ can approach a limit at high concentrations of complexing agent:

$$\lim_{C_b \to \infty} k_{app.} = \frac{2k_e \cdot K' \cdot PC_b \cdot PC_a}{K} \qquad \text{(Eq. A11)}$$

For example, $k_{app.}$ approaches 20×10^{-3} in curve B, Fig. 9, at high concentrations of complexing agent. Complexing agents which form complexes with large K' and/or have a large partition coefficient will tend to increase the apparent permeability of the barrier toward A while the opposite is true for complexing agents with large K and/or small partition coefficient.

If $k_{app.}$ is determined at several concentrations of complexing agent, the diffusion coefficient of A and E in the membrane can be determined. Although Eq. A10 can be used for this purpose, it is more convenient to define PC_a^*, the apparent barrier phase–aqueous phase partition coefficient of A:

$$PC_a^* = \frac{C_a' + C_e'}{C_a + C_e} = PC_a \cdot \frac{1 + K' \cdot PC_b \cdot C_b}{1 + K \cdot C_b} \qquad \text{(Eq. A12)}$$

From Eqs. A10 and A12, it follows that:

$$k_{app.} \cdot (1 + K \cdot C_b) = \frac{2F \cdot PC_a \cdot (D_a - D_e)}{L} + \frac{2F \cdot D_e \cdot PC_a^* \cdot (1 + K \cdot C_b)}{L} \qquad \text{(Eq. A13)}$$

where F is the ratio of the surface area of the barrier to the volume of the source compartment. Equation A13 predicts that a plot of $k_{app.}(1 + K \cdot C_b)$ *versus* $PC_a^*(1 + K \cdot C_b)$ will be linear and the intercept will be different from zero if D_a is not equal to D_e. Both diffusion coefficients can be calculated from the slope and intercept of such a plot if the dimensions of the barrier and the volume of the source solution are known. Use of Eq. A13 rather than a rearranged form of Eq. A10 to calculate the diffusion coefficients obviates the need for knowing K' or PC_b; and when $K \cdot C_b \ll 1$, it is not necessary to know C_b. Comparison of Eqs. A10 and A12 shows that the effect of a complexing agent on the overall absorption rate constant ($k_{app.}$) and on the apparent partition coefficient (PC_a^*) of A will not be parallel except when k_a equals k_e.

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