Effect of Molecular Interaction on Permeation of Organic Molecules Through Dimethyl Polysiloxane Membrane

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Abstract □ In order to investigate the nature of permeation of organic molecules through a nonpolar membrane in the presence of other molecular species, some model studies have been made with *p*-nitrophenol as an ionizable diffusate and *N,N*-dialkylamides and alkylxanthines as complexing agents. Effect of diethylpropionamide on permeation of salicylic acid was also examined. 8-Methoxycaffeine and caffeine were used as model compounds for nonionizable molecules and permeability constants were determined with and without complexing agents. The permeability of a diffusate increased in the presence of complexing agents which interact strongly with the diffusate in nonpolar environments, while it decreased in the case of the agents which complex with the diffusate mainly in aqueous solution.

Keyphrases □ Molecules, organic—dimethyl polysiloxane membrane permeation □ Membrane permeation, organic molecules—molecular interaction effect □ Ionizable diffusates-complexing agents—membrane permeation □ Colorimetric analysis—spectrophotometer □ UV spectrophotometry—analysis □ GLC—analysis

Extensive studies by Garrett and Chemburkar on diffusion of drug molecules through a silicone rubber membrane (1–3) have contributed much to knowledge on drug diffusion through a polymeric membrane. Because of the nonpolar nature of silicone rubber materials (4), a dimethyl polysiloxane membrane would be useful for investigation of effects of molecular interaction on drug permeation through a membrane. The first objective of the present investigation was to examine the nature of permeation of molecules through a nonpolar membrane in the presence of some other molecular species. This would provide additional information concerning the effect of complex formation on drug absorption which has been studied by Levy and his associates (5, 6).

The second objective was to explore some possibilities of controlling rate of permeation of organic molecules through a membrane by physicochemical means. In view of possible application of silicone rubber materials to some dosage forms (7, 8), it would be worthwhile to examine how the rate of permeation of molecules through a membrane can be modified.

EXPERIMENTAL

Materials—Dimethyl polysiloxane[1] sheeting in a labeled thickness of 5 mil was used throughout this study. *p*-Nitrophenol[2], salicylic acid[3], 8-methoxycaffeine[4] (recrystallized from methanol-carbon tetrachloride), and caffeine[5] (recrystallized from water) were used as model diffusates. Other compounds employed as complexing agents are also commercially available chemicals and were purified whenever necessary (9).

Diffusion Studies—The quasi steady-state diffusion cell described by Garrett and Chemburkar (1) was used with the following modifications. One polytetrafluoroethylene[6] O-ring was used instead of two silicone rubber gaskets and the diameter of the area available for diffusion was 32 mm. The cell was initially equilibrated overnight in a shaker bath maintained at 30° with 100 ml. of distilled water in both arms. Subsequently water was removed by suction and 50 ml. of 0.005 *N* sodium hydroxide (for *p*-nitrophenol and salicylic acid) or water (for 8-methoxycaffeine and caffeine) was added to one arm and an equal volume of test solution was placed in the other arm.[7] All the solutions were prewarmed to 30°. In order to suppress the dissociation of *p*-nitrophenol and salicylic acid, their solutions were prepared in 0.001 *N* and 0.004 *N* hydrochloric acid, respectively. The cell was mechanically shaken horizontally at a rate of 148 ± 2 strokes/min.

Analytical Methods—*p-Nitrophenol*—A 0.5-ml. aliquot of a desorbing solution was pipeted into a 10-ml. volumetric flask, then 5 ml. of 0.01 *N* sodium hydroxide was added to it, and the mixture was made up to volume with water. The absorbance of this solution was recorded on a spectrophotometer[8] at the wavelength of maximum absorbance, 400 mμ. No complexing agent was found to interfere with the assay at this wavelength.

Salicylic Acid—A 0.5-ml. aliquot from the desorbing solution was acidified with 2 ml. of 0.1 *N* hydrochloric acid to suppress the dissociation and made up to volume (10 ml.) with water. The concentration of the resultant solution was spectrophotometrically determined at 302 mμ. Diethylpropionamide did not interfere with the assay.

8-Methoxycaffeine—A 1-ml aliquot was pipeted out of both desorbing and diffusing solutions. When methyl nicotinate was used as a complexing agent, absorbance at both 262 and 281 mμ was recorded and the content of each component was calculated by the standard method of simultaneous spectrophotometric analysis (10). When tryptophan was used as a complexing agent, 8-methoxycaffeine was extracted into carbon tetrachloride. A 1-ml. portion of carbon tetrachloride solution was then appropriately diluted with methanol and the xanthine was spectrophotometrically determined at 281 mμ. In the presence of phenols and *p*-hydroxybenzoic acid, the sample solutions were made alkaline with sodium hydroxide (0.005 *N* with respect to the final concentration) and 8-methoxycaffeine was then extracted into carbon tetrachloride.

Caffeine—A 1-ml. aliquot was pipeted out of both desorbing and diffusing solutions. The concentration of caffeine in the presence of salicylamide was determined in the following way. The sample solution was made alkaline with sodium hydroxide (0.005 *N* with respect to the final concentration) and the concentrations of salicylamide were determined at 328 mμ. The concentration of caffeine was then calculated from absorbance at 272 mμ and correction was made for absorbance due to salicylamide at the same wavelength. The concentration of caffeine in the presence of *p*-bromophenol was determined in the following way. To a 1-ml. aliquot was added 9 ml. of 0.1 *N* sodium hydroxide and the resultant solution was shaken with 2 ml. of chloroform. One milliliter of the organic layer was pipeted out and absorbance at 272 mμ was measured after appropriate dilution with methanol.

Determination of Partition Coefficient—Five-milliliter portions of sample solutions were shaken with a 5-ml. portion of carbon

[1] Silastic, Medical Products Div., Dow Corning Corp., Midland, Mich.
[2] Fisher reagent grade, Chemical Manufacturing Div., Fisher Scientific Co., Fair Lane, N. J.
[3] Baker & Adamson reagent, General Chemical Div., Allied Chemical, New York, N. Y.
[4] Eastman Organic Chemicals, Div. of Eastman Kodak Co., Rochester, N. Y.
[5] British Drug Houses, Toronto, Canada.
[6] Teflon, E. I. du Pont de Nemours & Co., Wilmington, Del.
[7] In this paper a solution containing diffusate at time zero is referred to as a diffusing solution while the other side of the membrane is designated as a desorbing solution.
[8] Beckman Model DB, Beckman Instruments, Fullerton, Calif.

tetrachloride by means of an aliquot shaker[9] in a low temperature incubator[10] (25°) for about 2 hr. The concentrations of aromatic compounds remaining in the water layer were determined as described in the *Analytical Methods*. The concentrations in carbon tetrachloride were assumed to be equal to the difference between the initial concentration in water and the concentration in water after extraction. The concentrations of aliphatic molecules remaining in the water layer were determined by GLC. A gas chromatograph[11] equipped with a dual flame ionization detector and a 0–10 mv. recorder[12] was employed. The chromatographic column was a 0.31-cm. (0.125-in.) o.d. stainless steel column, 1.83 m. (6 ft.) in length and packed with 10% silicone elastomer[13] on a diatomite[14] (80–100 mesh). The column was silanized *in situ* with hexamethyldisilazane and equilibrated overnight at the operating conditions; oven temperature 140°; injection port temperature, 301°; detector temperature, 283°; hydrogen pressure 15 lb./sq. in.; air pressure, 25 lb./sq. in., helium pressure, 40 lb./sq. in. (80 ml./min.). Either dimethylacetamide or diethylpropionamide was used as an internal standard.

RESULTS AND DISCUSSION

Ionizable Diffusates—*p*-Nitrophenol and salicylic acid, with pKa values (11) of 7.1 and 3.0 were selected as model ionizable compounds. The concentration of diffusible species in the desorbing solution can be kept to a zero value by maintaining diffused molecules in a dissociated form. During the first 4 to 5-hr. period of the permeation experiment, the drug concentration of the diffusing solution is not altered significantly; and it may be assumed that the concentration of a diffusate during this period is equal to its initial concentration, C_0, in the diffusing solution. For such a system the following equation modified from that derived by Garrett and Chemburkar (2) for a steady-state diffusion can be employed

$$C_2 = k_p \frac{AC_0t}{V_2} \qquad \text{(Eq. 1)}$$

where C_2 = concentration of diffusate in desorbing solution, A = area of the membrane, V_2 = volume of the desorbing solution, and k_p = permeability constant, which is the product of diffusivity of the drug in the membrane and its membrane/water partition coefficient divided by thickness of the membrane. Plots of C_2 *versus* t will give a straight line with slope = $k_p \times AC_0/V_2$ from which k_p can be computed.

Effect of Complexing Agents on Permeation of p-Nitrophenol—A typical set of data for the permeation of *p*-nitrophenol in the presence and absence of complexing agents are plotted in Fig. 1 in accordance with Eq. 1. Depending on the nature of the complexing agent, two opposing effects on the rate of permeation were observed. Accordingly, the rate of permeation increased in the presence of diethylpropionamide while it decreased in the presence of caffeine. In order to explore the observed effects, the rate of permeation of *p*-nitrophenol was determined in the presence of a number of complexing agents. The data for this study were plotted in the same way as illustrated in Fig. 1. All the results gave linear relationships of C_2 *versus* t. The apparent permeability constants, k_p, were computed from the slopes of the lines and they are summarized in Table I. If one compares the effect of amides on the k_p values, it can be observed from the table that effect on the permeation varies among the *N,N*-dialkylamides and that it is concentration dependent, k_p value increasing with increase in concentration of diethylpropionamide.

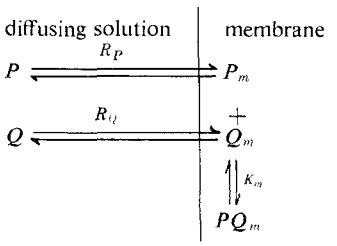
In order to explain the difference in the drug permeability in the presence of the amides, apparent partition coefficients (CCl_4/H_2O) of the amides as well as of *p*-nitrophenol in the presence of the amides were determined and these results are summarized in the third and fifth columns of Table I. In view of approximately the same hydrogen bonding ability of the amides with *p*-nitrophenol in a nonpolar solvent (12), the observed difference in the apparent partition coefficients of *p*-nitrophenol can be related to the difference

Table I—Apparent Permeability Constants, k_p, and Apparent Partition Coefficients, R, of *p*-Nitrophenol in the Presence of Complexing Agents

Complexing Agent	Concn., m*M*	R[a]	*p*-Nitrophenol— $k_p \times 10^2$ cm./hr.	*p*-Nitrophenol— $R_{app.}$[b]
None	—	—	6.55	0.086
Dimethylacetamide	40	0.00	6.55	0.18
Dimethylpropionamide	40	0.15	7.27	0.73
Diethylacetamide	40	0.35	8.45	1.9
Diethylpropionamide	40	2.1	12.8	4.2
	20		9.98	
	10		8.51	
Dioxane	40	0.74	6.52	0.13
n-Amyl alcohol	40	2.3	7.63	0.20
Dimethyl sulfoxide	40	0.031	6.55	0.086
Theophylline	10	0.002	5.74	0.086
Caffeine	10	0.21	5.59	0.097
8-Methoxycaffeine	10	5.5	5.48	0.26

[a] CCl_4/H_2O at 25°. [b] CCl_4/0.001 *N* HCl at 25°.

in the partition coefficients of the amides themselves. This point is elaborated by the following scheme and equation:



$$R_{app.P} = \frac{P_m + PQ_m}{P} = \frac{P_m(1 + K_mQ_m)}{P} = R_P(1 + K_mQ_m) \qquad \text{(Eq. 2)}$$

where P and Q represent equilibrium concentrations of *p*-nitrophenol and an amide, respectively. Rx is a partition coefficient of species X, K is a stability constant, and the subscript m stands for "in the membrane." According to Eq. 2, the apparent partition

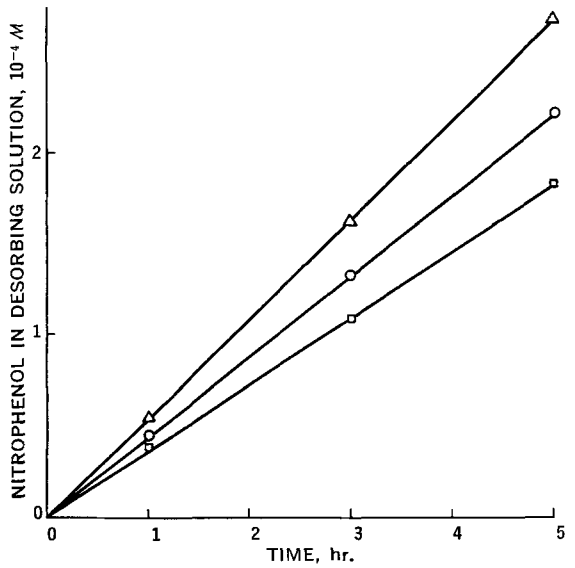


Figure 1—*Permeation of* p*-nitrophenol in the presence of 10 m*M *caffeine* (□) *or 10 m*M *diethylpropionamide* (△) *and in the absence of complexing agent* (○) *at 30°. The diffusing solution was 50 ml. of 4 m*M p*-nitrophenol in 0.001* N *hydrochloric acid with or without complexing agent and the desorbing solution was 50 ml. of 0.005* N *sodium hydroxide solution.*

[9] Lab-Tek, Ames Lab-Tek, Inc., Westmont, Ill.
[10] Model 82, Fisher Scientific Co.
[11] F & M Model 700, F & M Scientific Div., Hewlett Packard, Palo Alto, Calif.
[12] Speedomax W, Leeds & Northrup Co., Philadelphia, Pa.
[13] W98, F & M Scientific Div., Hewlett Packard, Palo Alto, Calif.
[14] Diatoport S, F & M Scientific Div., Hewlett Packard, Palo Alto, Calif.

Table II—Effect of Diethylpropionamide (DEP) on Permeability Constant, k_p, of *p*-Nitrophenol

Location of DEP	$k_p \times 10^2$, cm./hr.
(Absent)	6.55
Diffusing solution[a]	12.8
Desorbing solution[a]	7.37
Both diffusing and desorbing solutions[a]	13.8
Membrane[b]	6.91

[a] 40 m*M* DEP. [b] The membrane was presoaked with 50 ml. each of 100 m*M* DEP in both arms for 16 hr.

Table IV—Apparent Permeability Constants, k_p, and Apparent Partition Coefficients, *R*, of Caffeine and Salicylamide

Diffusate[a]	Complexing Agent	$k_p \times 10^2$, cm./hr.	R^c
Caffeine	None	0.216	0.21
Caffeine	*p*-Bromophenol[b]	0.228	0.72
Caffeine	Salicylamide[a]	0.148	0.13
Salicylamide	None	2.57	0.14
Salicylamide	Caffeine[a]	2.17	0.098

[a] 16 m*M*. [b] 32 m*M*. [c] CCl_4/H_2O at 25°.

coefficient of the phenol depends on the values of R_P, K_m, and Q_m. Since the partition coefficient of uncomplexed phenol, R_P, is constant, and the stability constants of hydrogen bonded complex, K_m, are about the same for the *N,N*-dialkylamides (12), the apparent partition coefficient is primarily a function of the amide concentration in the membrane. Thus amides with greater partition coefficients gave greater apparent partition coefficients of *p*-nitrophenol as seen in Table I.

In order to evaluate the role of diethylpropionamide in the transfer of the phenol across the membrane, diethylpropionamide was placed unilaterally in the diffusing solution, in the desorbing solution, and in the membrane; and bilaterally in both the diffusing and desorbing solutions. These data, as given in Table II, showed that the amide substantially increased the permeability constant of the phenol only when it was placed in the diffusing solution. From these results it is highly improbable that the amide merely changes the permeation characteristic of the membrane (diffusivity of a diffusate in the membrane). The increased permeation of *p*-nitrophenol may be attributed to the increase in concentration of *p*-nitrophenol in the membrane at its interface with diffusing solution or in other words, to the increase in apparent partition coefficient. The greater apparent partition coefficient of *p*-nitrophenol in the presence of diethylpropionamide may be rationalized by the greater partition coefficient of the amide itself and the presence of hydrogen bonding interaction in the nonpolar medium between the phenol and the amide (13). Dimethylacetamide, on the other hand, though it is as good a proton acceptor as diethylpropionamide, does not partition into a nonpolar solvent, and consequently has insignificant effect on the partition coefficient of the *p*-nitrophenol, thus supporting the importance of partitioning prior to diffusion through the membrane.

A possible alternative mechanism includes faster diffusion of the *p*-nitrophenol-amide complex through the membrane than uncomplexed *p*-nitrophenol. This is highly unlikely since the stability constant of such a complex in a bulk aqueous solution was found to be very small (12).

The results showing the influence of dioxane, *n*-amyl alcohol, dimethyl sulfoxide, and xanthines on rate of permeation of *p*-nitrophenol are also included in Table I. Although dioxane and *n*-amyl alcohol partiton into the nonpolar solvent to an appreciable extent, they failed to modify the permeability constant of *p*-nitrophenol in any substantial way. This is in accord with the small increase in partition coefficients of *p*-nitrophenol in the presence of these molecules. The weak proton acceptor property of ethers and alcohols (14) may explain the present observation; namely, K_m in Eq. 2 is so small that $R_{app.P}$ does not differ significantly from R_P. Dimethyl sulfoxide, on the other hand, is reported to be a strong proton acceptor (15). The failure of the sulfoxide to modify the permeability can be attributed to its small partition coefficient. Thus a large partition coefficient, together with the strong hydrogen acceptor property of the complexing agent appear to be the factors responsible for accelerated permeation of *p*-nitrophenol.

Table III—Apparent Permeability Constants, k_p, and Apparent Partition Coefficients, *R*, of 8-Methoxycaffeine in the Presence of Complexing Agents

Complexing Agent	Concn., m*M*	8-Methoxycaffeine: *R*[a]	8-Methoxycaffeine: $k_p \times 10^2$, cm./hr.	8-Methoxycaffeine: $R_{app.}$[a]
None	—	—	3.73	5.4
p-Chlorophenol	60	2.1	4.11	49
p-Nitrophenol	10	0.097	3.51	5.2
Methyl nicotinate	60	5.3	2.17	5.2
Tryptophan	24	0.0	2.29	2.7
p-Hydroxybenzoic acid	30	0.018	2.02	1.8

[a] CCl_4/H_2O at 25°.

Alkylxanthines in general reduced the apparent permeability coefficients of *p*-nitrophenol. *p*-Nitrophenol interacts with alkylxanthines both in aqueous and nonpolar solutions (12). In the case of theophylline with a very minor partitioning into the nonpolar solvent, its complexation with *p*-nitrophenol in aqueous solution might be the sole cause of the reduction in the permeability. The retarding effect of 8-methoxycaffeine was not found to be as pronounced as expected from its great complexing tendency (16) since the xanthine partitions into a nonpolar solvent to an appreciable extent.

In spite of an increase in the apparent partition constant of *p*-nitrophenol in the presence of alkylxanthines, its permeability decreased. For example, the permeability constants of caffeine and 8-methoxycaffeine are 0.216×10^{-2} cm./hr. and 3.73×10^{-2} cm./hr., while their partition coefficients are relatively large, namely, 0.21 and 5.4 (see Tables III and IV). This discrepancy may have resulted from the fairly small diffusivity of alkylxanthines. It should be noted here that permeation is a kinetic phenomenon whereas partition coefficients are equilibrium constants.

Effect of Diethylpropionamide on Permeation of Salicylic Acid—The permeation behavior of salicylic acid in the presence and absence of diethylpropionamide is depicted in Fig. 2. The partition coefficients of salicylic acid at a 4 m*M* concentration in the absence and presence of the amide (40 m*M*) are 0.59 and 5.7, respectively. Thus, the increased permeation of salicylic acid in the presence of diethylpropionamide may be attributed to its increase in the partitioning property.

Nonionizable Diffusates—8-Methoxycaffeine and caffeine were chosen as model nonionizable molecules. For these drugs the following equation developed by Garrett and Chemburkar for quasi steady-state diffusion is applicable.

$$\log \frac{C_0}{C_1 - C_2} = \frac{0.869 k_p A}{V_2} t \qquad \text{(Eq. 3)}$$

wherein C_1 is the concentration of diffusate in a diffusing solution and all other terms are as previously defined in Eq. 1. A plot of log $C_0/(C_1 - C_2)$ *versus* t will yield a straight line and the permeability constant can be calculated from the slope of the line.

Effect of Complexing Agents on Permeation of 8-Methoxycaffeine—The permeation data for 8-methoxycaffeine in the presence and absence of tryptophan or *p*-chlorophenol are plotted in Fig. 3. The apparent permeability constants obtained from these plots and from similar plots with other complexing agents are compiled in Table III together with pertinent partitioning data. In the case of phenols, *p*-nitrophenol reduced the permeability constant of 8-methoxycaffeine, while *p*-chlorophenol increased it. Since both of these phenols interact with the xanthine in aqueous as well as in non-

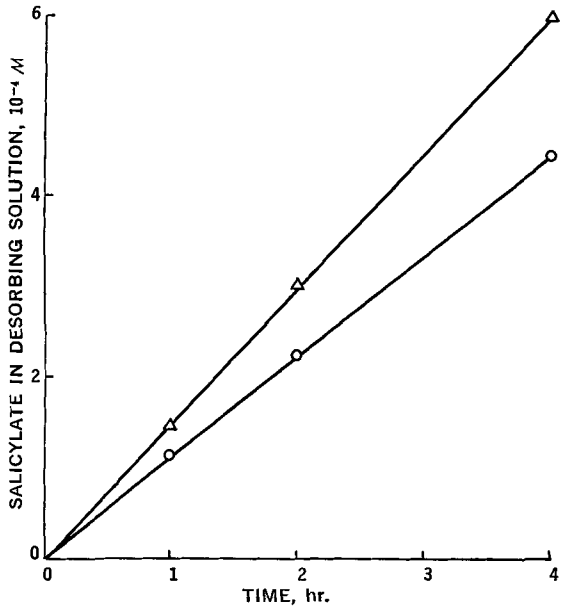


Figure 2—*Permeation of salicylic acid in the presence* (△) *and absence* (○) *of diethylpropionamide at 30°. The diffusing solution was 50 ml. of 4 mM salicylic acid in 0.004* N *hydrochloric acid solution with or without 40 mM diethylpropionamide and the desorbing solution was 50 ml. of 0.005* N *sodium hydroxide solution.*

polar environment (12), two counteracting effects may take place for the present systems. Complexation in aqueous solution would reduce the permeability constant of 8-methoxycaffeine while interaction at the membrane interface with the diffusing solution would increase it. With *p*-nitrophenol, whose partition coefficient is small, complexation with 8-methoxycaffeine in aqueous solution is a dominant factor, while for *p*-chlorophenol, with a large partition coefficient, hydrogen bonding interaction at the interface outweighs the complexation effect in an aqueous solution, resulting in a net increase in the permeabiliy constant.

Methyl nicotinate (Table III) was observed to lower the permeation of 8-methoxycaffeine. Although methyl nicotinate has a large partition coefficient, it has no hydrogen bonding affinity for the xanthine in a nonpolar environment (12). Therefore complexation in the aqueous solution may account for the reduction in the

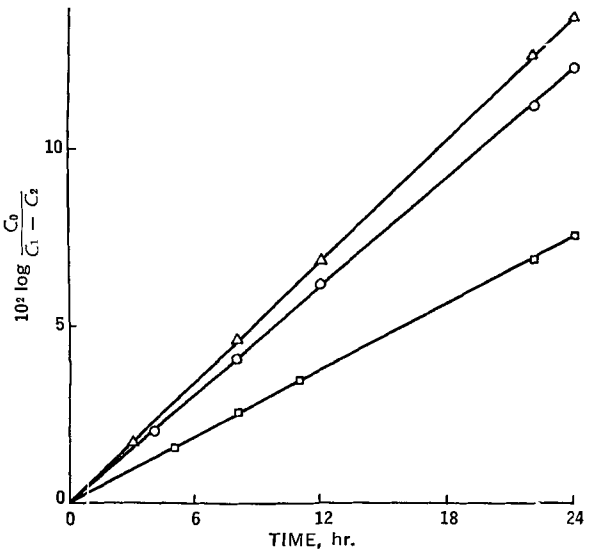


Figure 3—*Permeation of 8-methoxycaffeine (4 mM) in the presence of 24 mM tryptophan* (□) *or 60 mM p-chlorophenol* (△) *and in the absence of complexing agent* (○) *at 30°.*

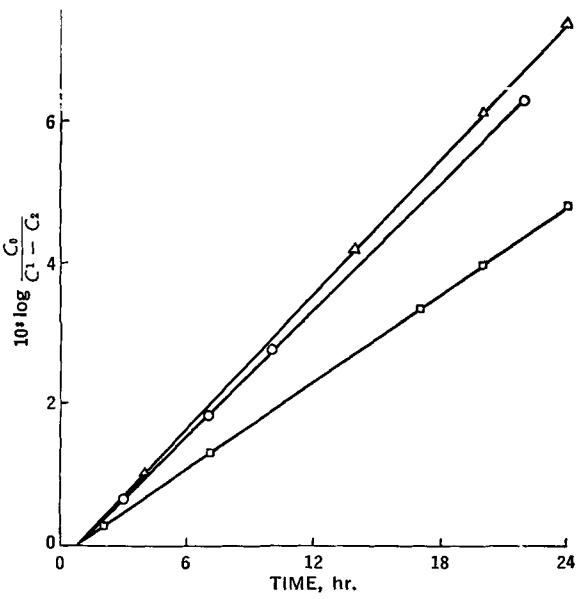


Figure 4—*Permeation of caffeine (16 mM) in the presence of 16 mM salicylamide* (□) *or 32 mM p-bromophenol* (△) *and in the absence of complexing agent* (○) *at 30°.*

permeation of the xanthine. The apparent partition coefficients of 8-methoxycaffeine are reduced due to the presence of tryptophan and *p*-hydroxybenzoic acid. These complexing agents are not partitioned into the nonpolar solvent as shown in Table III. Thus the decrease of the permeability constants of 8-methoxycaffeine by tryptophan and *p*-hydroxybenzoic acid can be inferred as being due to the complexation in the aqueous solution.

Effect of Salicylamide and p-Bromophenol on Permeation of Caffeine—Data for the permeation of caffeine with and without complexing agents are presented in Fig. 4. The permeation and partitioning results for caffeine and salicylamide are given in Table IV. It should be noted in Fig. 4 that the permeation rate plots yielded an intercept on the abscissa, indicating an apparent time lag. Comparing the k_p value of caffeine with those of other drugs this would be expected, since caffeine gave the lowest value. This figure also illustrates that the rate of permeation of caffeine was accelerated by *p*-bromophenol and it was decelerated by salicylamide. The results may be rationalized in the same way as for 8-methoxycaffeine. For example, with salicylamide whose partition coefficient is small (R = 0.14), complexation in an aqueous solution may be considered to have a dominant effect, while with *p*-bromophenol whose partition coefficient is large (R = 3.14), interaction at the interface would outweigh the complexation in an aqueous solution. The permeability constant of salicylamide was also found to decrease in the presence of caffeine. These observations can be substantiated by the measured values of partition coefficient of the diffusate in the presence of the complexing agents. Experimentally obtained values are summarized in Table IV.

CONCLUSIONS

This study illustrates that the rate of permeation of a drug can be increased or decreased in the presence of a complexing agent, and the magnitude of this effect is dependent upon the physicochemical nature of the agent. From the results it may be concluded that the agents which complex with a diffusate (*a*) only in aqueous solution would reduce the permeability constant; (*b*) both in aqueous and nonpolar environments would either decrease or increase it depending upon the partitioning behavior of the complexing agents and the relative stability constants of the complex in both environments; and (*c*) only in nonpolar environment would increase it.

If a drug in question is an acidic molecule, a complexing agent of an aliphatic proton acceptor type, with a fairly large partition coefficient and favorable toxicological characteristics, would be a candidate for an agent which may be used to enhance the permeation of the drug across biological membranes.

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Factors Affecting the Synthesis of Penicillinase by Staphylococcus Aureus

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Abstract □ The effects of aeration, ferric ions, porphyrins, and disodium versenate were studied on the production of penicillinase (ps) by *Staphylococcus aureus* 55-C-1 in synthetic medium. The production of ps was twice as high in shaken compared to static cultures although growth ultimately was the same. Ferric ions in a concentration of 2.5×10^{-4} *M* had some stimulatory effect on ps production in static cultures but caused much greater ps stimulation in shaken cultures. This stimulatory effect could be demonstrated only if ferric ions were added prior to 3 hr. of incubation. Hemin in a concentration of 2.5×10^{-5} *M* depressed ps production in the presence of ferric ions but one-tenth the concentration of hemin did not. Hematin in the same concentration was without effect. Protoporphyrin in a concentration of 2.5×10^{-5} *M* inhibited growth in the absence of ferric ions and depressed ps production in the presence of ferric ions. One-tenth the concentration of protoporphyrin had no effect on growth but depressed ps production and only slightly depressed ps production in the presence of ferric ions. A concentration of 2.5×10^{-6} *M* hematoporphyrin inhibited ps production in the presence of ferric ions when the former was added early in the growth curve. Disodium versenate in a concentration of 1.32×10^{-5} *M* in the presence of ferric ions permitted good growth but no ps production.

Keyphrases □ Penicillinase production—*Staphylococcus aureus* □ Aeration, shaking effect—penicillinase production □ Porphyrins, ferric ions, disodium versenate, effect—penicillinase production □ Optical density—*S. aureus* growth determination □ Manometric determination—penicillinase

Abraham and Chain (1) were the first to show that certain bacteria produce an enzyme capable of hydrolyzing penicillin. Since the latter paper was published in 1940, a number of workers have demonstrated that the resistance of strains of *Staphylococcus aureus* to penicillin was related to the production of penicillinase, the enzyme which was shown to hydrolyze penicillin. Although in some studies penicillin resistance was demonstrable in the absence of penicillinase production (2, 3), there seems to be good correlation between the ability of staphylococci to produce penicillinase and to be resistant to the action of penicillin both *in vitro* and *in vivo* (4–6). Penicillinase production has been similarly implicated in penicillin resistance of *Bacteroides* sp. (7).

In some microorganisms, penicillinase has been found to be an inducible enzyme and many studies (8–12) have concentrated on the nature of the induction of penicillinase elaboration. Some strains of *S. aureus* produced penicillinase constitutively (13, 14) and a number of workers have been concerned with the conditions and factors affecting constitutive production of penicillinase. It was felt that if the factors which control or affect penicillinase production in a strain of *S. aureus* producing penicillinase constitutively could be identified, it was possible that this knowledge could be applied ultimately in the treatment of an infection caused by a penicillinase-producing strain of *S. aureus*. That is, a strain which otherwise would be resistant to penicillin could be made susceptible to the action of penicillin if penicillinase production could be inhibited by the concurrent administration of a nontoxic