Interaction of Pharmaceuticals with Schardinger Dextrins VI

Interactions of β-Cyclodextrin, Sodium Deoxycholate, and Deoxycholic Acid with Amines and Pharmaceutical Agents

By JOHN L. LACH and WAYNE A. PAULI*

Data are presented for the interactions of β-cyclodextrin and sodium deoxycholate with 11 pharmaceutical agents in aqueous solution. Three of the compounds were also studied as to possible reactivity with deoxycholic acid. Interactions were observed with all of the systems studied. Similar solubility isotherms were obtained for the three pharmaceuticals when interacted with both deoxycholic acid and its sodium salt indicating possible similarities in the reaction mechanisms for these two complexing agents. The larger molecules showed a lower degree of interaction with both β-cyclodextrin and sodium deoxycholate, indicating the importance of molecular size and structure for optimum reactivity. Stoichiometries could be determined for several of the interactions due to the presence of plateau regions in their solubility isotherms. Formation constants and free energies of formation are also reported for a number of the insoluble complexes.

THE IMPORTANCE of molecular complex formation in pharmaceutical formulation is certainly evident. Applications of this type of interaction can be made in the area of drug solubilization, stabilization, etc. The problem of stabilization is of particular interest in that numerous attempts have been made to retard degradation of pharmaceutical compounds. These include pH considerations, changes in solvent systems, antioxidants, and complex salt formation. The use of molecular complex formation in this area represents a relatively new approach to this problem of stability (1–5). Complexation by means of inclusion formation has been used successfully in the stabilization of labile drugs such as benzocaine, vitamin A, and various fatty acids (6, 7). This mode of protection for an unstable molecule offers definite advantages in that the entire molecule can be shielded from its surroundings. It has also been recognized that complexes not only influence the stability and appearance of pharmaceutical preparations, but may also exert some influence on the pharmacological and even biochemical mechanisms by which they operate.

The previous paper of this series dealt with a study of the interactions of β-cyclodextrin with a series of phenyl-substituted carboxylic acids in an effort to explain more clearly the nature of cyclodextrin interaction in aqueous solution. It was the objective of this study to investigate and compare the interaction tendencies of various medicinal agents with both β-cyclodextrin and deoxycholic acid, as only a limited number of these interactions have been reported in the literature. The solubilizing effect of sodium deoxycholate on these drugs was also studied with the hope of understanding more fully the mechanism responsible for this phenomenon.

Received September 13, 1965, from the College of Pharmacy, University of Iowa, Iowa City.

Accepted for publication October 4, 1965.

Abstracted in part from a dissertation presented by Wayne A. Pauli to the Graduate College, University of Iowa, in partial fulfillment of Doctor of Philosophy degree requirements.

* Fellow of the American Foundation for Pharmaceutical Education. Present address: Ciba Pharmaceutical Co., Summit, N. J.

Previous paper: Pauli, W. A., and Lach, J. L., *J. Pharm. Sci.*, **54**, 1745(1965).

EXPERIMENTAL

Reagents.—β-Cyclodextrin $[\alpha]_D^{25°}$ in water = + 162.5 ±0.5; lidocaine,[1] m.p. 68–69°; adiphenine hydrochloride,[2] m.p. 113–114°; antazoline hydrochloride,[3] m.p. 234–236°; methapyrilene hydrochloride,[4] m.p. 161–163°; tripelennamine hydrochloride,[5] m.p. 189–192°; testosterone, m.p. 155–156°; morphine, m.p. 250°; procaine hydrochloride, m.p. 154–156°; meperidine hydrochloride, m.p. 187–189°; cortisone acetate, m.p. 235–238°; reserpine, m.p. 257–260°; sodium deoxycholate, analytical grade; deoxycholic acid, m.p. 171–172°; 1 *N* standard sodium hydroxide solution.

Apparatus.—The same apparatus described in the previous paper of this series was used in this study with the following addition: radiometer pH meter, type PHM4C.

Procedures.—The β-cyclodextrin was prepared by the procedure used previously in these laboratories (8). Complex formation was studied by means of the solubility method of Higuchi and Lach (9). The experimental procedures were similar to those outlined in the previous paper of this series, except for the following modifications.

With a few of the drugs, the free bases were formed in the reaction vials from the corresponding hydrochloride salts by the addition of a sufficient amount

[1] Marketed as Xylocaine by Astra Pharmaceutical Products, Inc., Worcester, Mass.

[2] Marketed as Trasentine by Ciba Pharmaceutical Products, Inc., Summit, N. J.

[3] Marketed as Antistine by Ciba Pharmaceutical Products, Inc., Summit, N. J.

[4] Marketed as Histadyl by Eli Lilly & Co., Indianapolis, Ind.

[5] Marketed as Pyribenzamine by Ciba Pharmaceutical Products, Inc., Summit, N. J.

of standard sodium hydroxide solution. A 10% excess of the standard solution was added to insure complete neutralization of the salts. In these particular systems, the amount of distilled water was reduced to compensate for the volume of standard base solution. When deoxycholic acid was used as the complexing agent, it was necessary to employ hydroalcoholic systems because of the acids limited solubility in water. The drugs were analyzed spectrophotometrically at the following wavelengths: lidocaine, 262.5 mμ; adiphenine, 258 mμ; antazoline 242 mμ; methapyrilene, 239 mμ; tripelennamine, 245 mμ; testosterone, 238 mμ; morphine, 285 mμ; procaine, 289 mμ; meperidine, 257 mμ; cortisone acetate, 238 mμ; reserpine, 268 mμ. Because of the concentrations used and corresponding dilutions prior to spectrophotometric analysis, no interference in the absorption characteristics of the compounds tested was observed due to the presence of the various complexing agents. The pH of each reaction vial was determined following its analysis, and no appreciable pH changes were observed in any of the systems investigated.

RESULTS AND DISCUSSION

Solubility isotherms representing the interactions of various drugs with β-cyclodextrin, deoxycholic acid, and sodium deoxycholate are shown in Figs. 1–12. Although several of the amines studied with these complexing agents are known to be relatively unstable at high pH, no appreciable degradation was observed for any of the compounds during the course of this investigation. Definite interactions were observed with all of the systems studied. Linear plots, indicating a first-order dependence of the interactions on the complexing agent concentration, were obtained in most cases. Smaller slopes were generally observed for interactions of both β-cyclodextrin and sodium deoxycholate with larger and thus more sterically hindered molecules. This finding is in agreement with results reported in an earlier article in this series (10). It should be pointed out here that definite relationships between interaction slopes and relative reactivities exist only if the stoichiometries of the various systems are the same. Since stoichiometries could not be evaluated for a number of these interactions due to the nature of the solubility isotherms, the comparison of slopes must be viewed with some reservation.

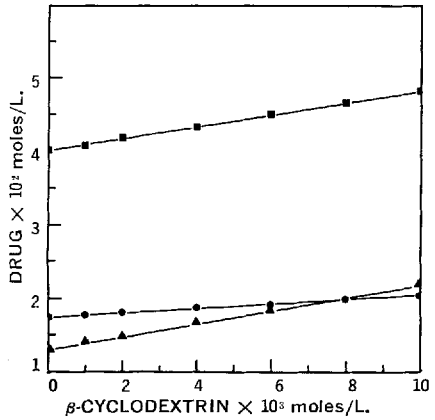

Fig. 1.—Interactions of procaine (■), lidocaine (●), and meperidine (▲) with β-cyclodextrin at 30°.

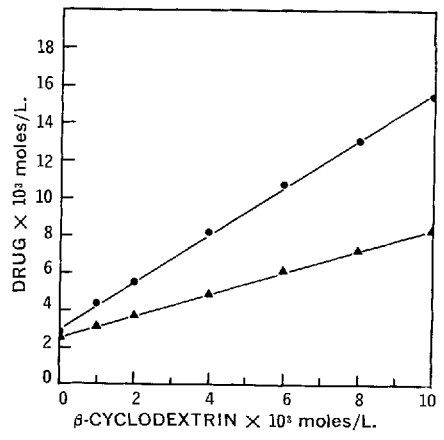

Fig. 2.—Interactions of antazoline (●) and methapyrilene (▲) with β-cyclodextrin at 30°.

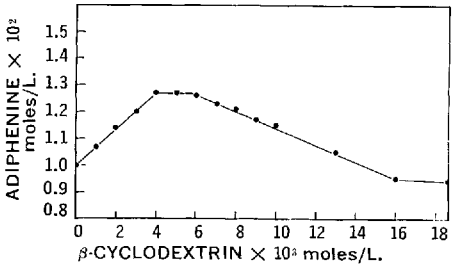

Fig. 3.—Interaction of adiphenine with β-cyclodextrin at 30°.

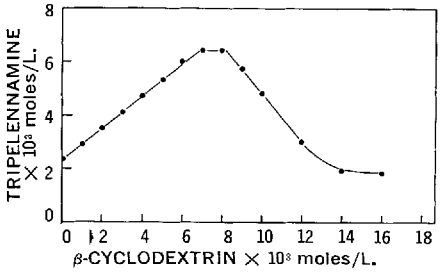

Fig. 4.—Interaction of tripelennamine with β-cyclodextrin at 30°.

Because of the hydroalcoholic systems employed for the deoxycholic acid studies, necessitated by the limited solubility of this acid in water, it is rather difficult to compare the interaction tendencies of this complexing agent with those of sodium deoxycholate and β-cyclodextrin. However, certain similarities were observed in the shapes of the solubility isotherms for both deoxycholic acid and its sodium salt.

β-Cyclodextrin Interactions.—A structural comparison of the compounds interacted with β-cyclodextrin reveals some interesting relationships. Referring to Table I, it can be observed that larger slopes are found for systems composed of β-cyclo-

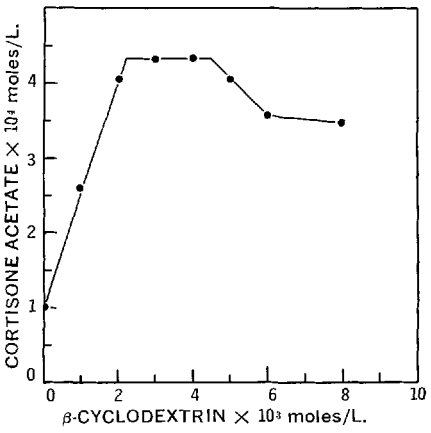

Fig. 5.—Interaction of cortisone acetate with β-cyclodextrin at 30°.

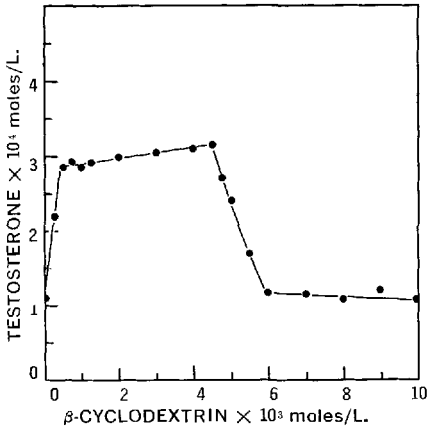

Fig. 6.—Interaction of testosterone with β-cyclodextrin at 30°.

Table I.—Slopes of Isotherms of Interactions of 11 Pharmaceutical Compounds with β-Cyclodextrin

Antazoline	1.25
Meperidine	0.88
Procaine	0.83
Adiphenine	0.68
Tripelennamine	0.60
Methapyrilene	0.59
Testosterone	0.44
Lidocaine	0.30
Morphine[a]	0.24
Cortisone acetate	0.15
Reserpine	0.001

[a] Data reported in *Reference 10.*

dextrin and small or medium-sized guest molecules containing one or more branched ring systems.

This increased reactivity resulting from the presence of a branched ring system might be attributed to the availability of a ring for either total or partial inclusion by the cyclodextrin. Therefore, inclusion, in combination with other attractive forces, would be expected to enhance the reactive tendency of a particular guest compound with β-cyclodextrin. If ring availability were assumed to be the primary factor for interaction with β-cyclodextrin, similar reactivities would be expected with both antazoline and adiphenine, since both molecules contain several branched phenyl groups. On comparison of the slopes for these compounds, listed in Table I, it can be seen that the slope representing the antazoline interaction is almost double that for the adiphenine system, again pointing toward a complex mechanism for these interactions. A smaller slope than would be expected for the interaction between lidocaine and β-cyclodextrin might be attributed to steric hindrance resulting from the multiple methyl group substitution on the phenyl ring. The bulky multi-ringed molecules including morphine, cortisone acetate, and reserpine show extremely small interaction slopes with β-cyclodextrin indicating only limited interactions for these systems. This would be expected due to the extreme size of the molecules and the resulting steric interference that would oppose their proper combination or fit with the cyclodextrin structure. It becomes obvious for molecules of this size, that interactions with β-cyclodextrin could not possibly occur by means of complete or true inclusion formation. The presence of reactive functional groups in these large molecules, capable of bonding with or of being partially included by the cyclodextrin, could be responsible for the observed interactions. Cortisone acetate and testosterone, because of structural similarity, would be expected to interact similarly with β-cyclodextrin. But on a comparison of the slopes for these interactions in Table I, a marked difference is observed with testosterone possessing the greater slope. As indicated previously, molecules of this size would obviously be too large to allow complete inclusion within the cyclodextrin cavity and therefore the mechanism might involve either interaction of or the enclosure of a functional group of the steroid by the cyclodextrin. The hydroxyl group in the C-17 position is relatively open and free in the testosterone structure, while the same group in the cortisone acetate molecule is in close proximity to a rather bulky side chain also at the C-17 position. Interference introduced by this group in the cortisone acetate structure could partially explain the decreased reactivity of this compound with β-cyclodextrin. Again, as emphasized previously, these interactions are, as expected, quite complex due to the size and complexity of the interacting species, and therefore it is quite conceivable that a number of factors are responsible for the net interactions observed.

Stoichiometries were calculated for those systems characterized by the presence of plateau regions in their solubility isotherms. These values, along with stoichiometries determined from an analysis of the isolated complexes, are found in Table II.

The formation constants, calculated in a manner

Table II.—Stoichiometries of the Drug–β-Cyclodextrin Complexes

Compd.	From Phase Diagram Drug–BCD	Analysis of Isolated Complex Drug–BCD
Cortisone acetate	1.98:1.00	2.05:1.00
Testosterone	2.03:1.00	2.06:1.00
Tripelennamine	1.01:1.00	1.08:1.00
Adiphenine	1.01:1.00	1.04:1.00

TABLE III.—FORMATION CONSTANTS AND FREE ENERGIES OF FORMATION OF DRUG–β-CYCLODEXTRIN COMPLEXES AT 30°

Compd.	K_f	K_f[a]	$F°$ (cal./mole)
Cortisone acetate	8.1×10^6	...	−9578
Testosterone	2.3×10^7	...	−10209
Tripelennamine	6.5×10^2	6.5×10^2	−3900
Adiphenine	2.1×10^2	2.1×10^2	−3220

[a] Values determined by the method of Thoma and Stewart for 1:1 complexes only.

analogous to those employed in previous studies (9), and the corresponding free energies of formation for these insoluble complexes are listed in Table III. A method proposed by Thoma and Stewart (11), involving a mathematically derived expression for the calculation of formation constants of 1:1 complexes, was applied to some of our interactions in an effort to check our calculations. These results were in good agreement with the formation constants determined from the solubility isotherms.

The magnitude of the apparent formation constants and free energies of formation observed for these insoluble complexes is indicative of the relative high degree of interaction. Formation constants for other systems involving β-cyclodextrin have been reported in the range of 10^2 to 10^4 (12, 13), again indicating the existence of very stable systems. These formation constants are quite high compared to the values of 1 to 100 reported in pharmaceutical interaction studies with complexing agents such as caffeine (9, 14). Thus interactions involving more than hydrogen bonding or dipole-dipole attractions are suspected, and a combination of these plus inclusion formation is a definite possibility.

Deoxycholic Acid and Sodium Deoxycholate Interactions.—The same compounds studied with β-cyclodextrin were also interacted with sodium deoxycholate in an attempt to clarify further the mechanism by which this agent exerts its solubilizing effects. Interactions were observed with all of the compounds and are represented by Figs. 7–10. No

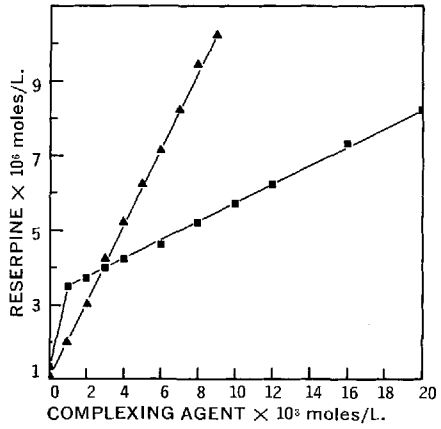

Fig. 7.—Interactions of reserpine with β-cyclodextrin (▲) and sodium deoxycholate (■) at 30°.

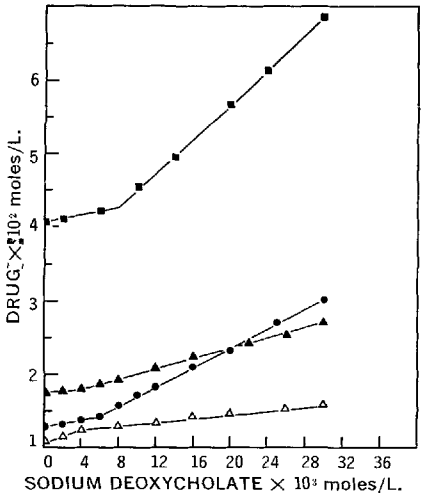

Fig. 8.—Interactions of procaine (■), lidocaine (▲), meperidine (●), and adiphenine (△) with sodium deoxycholate at 30°.

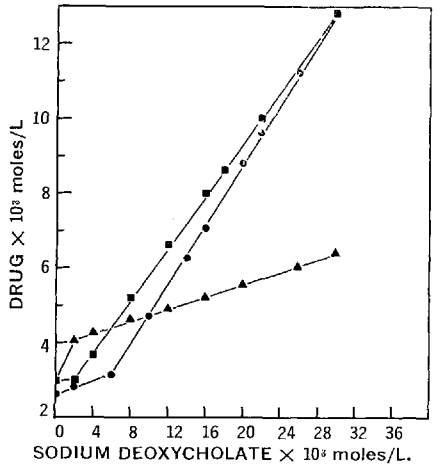

Fig. 9.—Interactions of methapyrilene (■), tripelennamine (●), and antazoline (▲) with sodium deoxycholate at 30°.

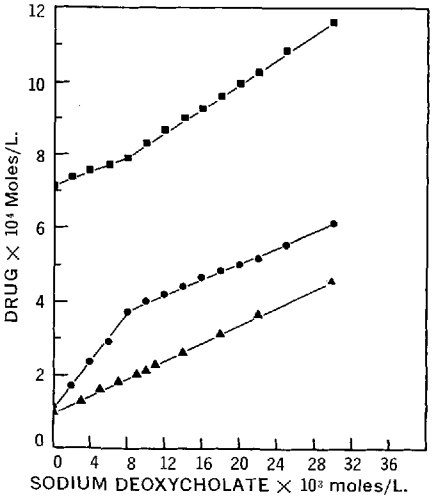

Fig. 10.—Interactions of morphine (■), testosterone (●), and cortisone acetate (▲), with sodium deoxycholate at 30°.

TABLE IV.—SLOPES[a] OF ISOTHERMS OF INTERACTIONS OF 11 PHARMACEUTICAL COMPOUNDS WITH SODIUM DEOXYCHOLATE

Procaine	1.18
Meperidine	0.66
Tripelennamine	0.40
Lidocaine	0.36
Methapyrilene	0.35
Adiphenine	0.13
Antazoline	0.08
Morphine	0.02
Cortisone acetate	0.01
Testosterone	0.01
Reserpine	0.00025

[a] The second slope was used when multislope isotherms were present.

plateau regions were found in the solubility isotherms for these interactions which prevented the determination of the exact stoichiometric relationships and the corresponding thermodynamic values. Slopes for interactions of these compounds with sodium deoxycholate are found in Table IV.

In general, as was true for the β-cyclodextrin interactions, the smallest compounds are seen to exhibit the greatest interaction slopes with sodium deoxycholate. This could either be due to the small size of the molecules or to their greater solubility, as one property is a corollary of the other. In most cases the interactions with β-cyclodextrin yielded larger slopes than the corresponding interactions with sodium deoxycholate, indicating a greater degree of complex formation with the cyclodextrin and possibly a different mechanism. Although there are certain similarities in the reaction isotherms observed, it should be pointed out that β-cyclodextrin is known to form monomolecular inclusion compounds, while a polymolecular mechanism, necessary in the build-up of the channel-like host structure, is essential for choleic acid inclusion formation.

Systems containing the amine bases and either of the complexing agents were quite alkaline (pH 9–12) due to the nature of the guest compounds themselves and to the excess sodium hydroxide added to insure complete liberation of the bases from their corresponding salts. While varying concentrations of sodium deoxycholate would be expected to have a measurable effect on the pH of neutral or acidic systems, no effect was observed in these investigations because of the elevated pH conditions employed. The pH changes observed in less basic systems ranged from 0.1–0.2 pH units. Even though this basic environment would not be expected to have any appreciable effect on β-cyclodextrin, it would markedly suppress hydrolysis of sodium deoxycholate resulting in a predominance of the deoxycholate anion in solution. Also in this pH range, the amine bases would exist as the nonprotonated form, and, therefore, the observed interactions would presumably be between the deoxycholate anion and the free bases. An acid–base reaction between a few of the strongly basic amines and deoxycholic acid in solution might be expected, but at this pH, the probability of an interaction of this type between the nonprotonated amine and the deoxycholate anion is small.

Multislope interaction isotherms obtained for a number of the sodium deoxycholate systems indicate the complexity of these molecular associations. At the present time, the popular theory is that sodium deoxycholate, acting as an anionic surfactant, exerts its solubilizing effects through micelle formation. There has been considerable evidence that lends support to this proposed mechanism (15–17). However, owing to the inability of isolating the reaction products for structural examination, the possibility of a mechanism similar to that for deoxycholic acid, involving channel-like inclusion formation, cannot be ruled out. Deoxycholic acid is believed to bond intermolecularly through the hydroxyl groups in the 3 and 12 positions forming a channel-like host structure. It is quite conceivable that the anion of this acid could assume a similar structure resulting in inclusion formation of the guest amines. Assuming that the carboxyl group of deoxycholic acid is also an active site for the intermolecular bonding necessary in the build-up of the host structure, one might expect a lesser degree of inclusion formation with the anion than with the undissociated acid. The complexity of these interactions could certainly point toward a mechanism composed of both inclusion formation and micellar solubilization. This combination might well account for the different slopes observed in the interaction isotherms for a number of the interactions that could also represent the formation of higher order complexes.

In an attempt to elucidate further the complex nature of sodium deoxycholate's mechanism of interaction, three of the compounds previously studied with this complexing agent were also interacted with deoxycholic acid. The interactions observed between each of the three compounds and deoxycholic acid are represented in Figs. 11 and 12. Hydroalcoholic systems were used for these interactions because of the acid's limited solubility in water. It is interesting to note that in these alcoholic systems, deoxycholic acid would exist almost entirely as the free acid, and, therefore, the concept of micellar solubilization would appear to be minimized as a possible mechanism since it has been

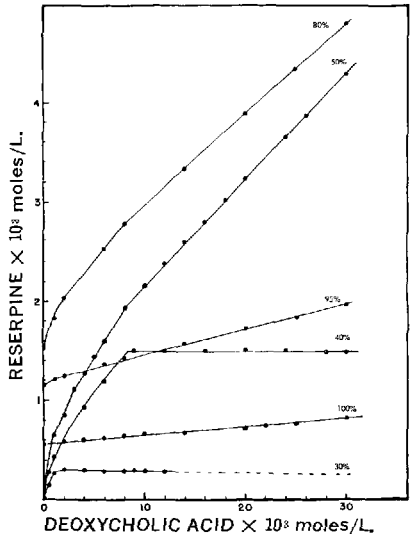

Fig. 11.—Interaction of reserpine with deoxycholic acid at 30° in hydroalcoholic systems.

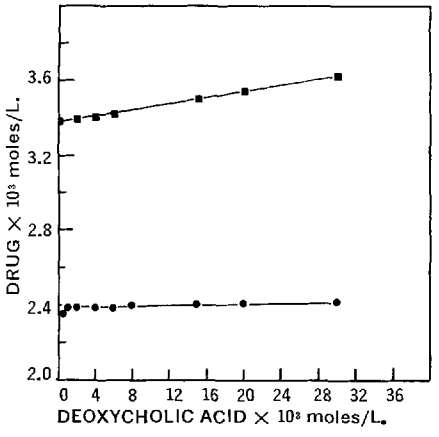

Fig. 12.—Interactions of cortisone acetate (■) (50% EtOH system) and testosterone (●) (30% EtOH system) with deoxycholic acid at 30°.

known for some time that micelles do not readily form in alcoholic solvents. A comparison of the slopes for these systems with the corresponding slopes for the sodium deoxycholate interactions is difficult because of the different solvent systems employed. However, the shapes of the solubility isotherms representing interactions of both the acid and its sodium salt do show certain similarities which might indicate a common mechanism for these complexing agents. Because of the unique multislope isotherm representing the reserpine–deoxycholic acid interaction in a 50% hydroalcoholic solvent, it was felt that a study of this system in solvents of varying alcoholic strength would be interesting, and might possibly yield a plateau region permitting the determination of the stoichiometry for this system. Therefore, this particular interaction was examined in alcoholic systems ranging from 0–100% ethanol. The corresponding isotherms representing the different alcoholic strengths are found in Fig. 11. As would be expected, the solubility pattern of the resulting complexes parallels the alcoholic concentration up to 80% ethanol, but a marked decrease in the interaction slopes is observed at higher alcoholic levels. The presence of deoxycholic acid is seen to exert a pronounced effect on the solubility of reserpine at the lower alcoholic concentrations employed over that of its solubility in the same alcoholic solutions without the deoxycholic acid. In the 50% alcoholic system, formation of the complex is seen to result in almost a fourfold increase in the solubility of reserpine. At lower alcoholic concentrations, the solubility of the complex appears to lie midway between the individual solubilities of reserpine and deoxycholic acid as would be expected. However, the marked decrease in solubility of the reserpine complex and interaction in 95% and absolute ethanol appears to parallel the unexpected decrease in solubility of the uncomplexed reserpine at these solvent concentrations. The solubility profiles of reserpine and deoxycholic acid in various hydroalcoholic systems are shown in Fig. 13. While deoxycholic acid is shown to be infinitely soluble at higher alcoholic concentrations, the solubility profile of reserpine is seen to possess a maximum in approximately 80% ethanol. Solubility studies involving a number of medicinal agents have pointed out the presence of such a maximum solubility in various cosolvent mixtures (18, 19), suggesting that a definite correlation exists between the solubility of the solute and the dielectric constant of the solvent system employed. The solubility of reserpine in these cosolvent systems involves solute-solvent and solvent-solvent associations, and since complexation of reserpine with deoxycholic acid would be in competition with these various associations, it becomes obvious that the mechanism involved in reserpine–deoxycholic acid interaction in these systems is complex. Since it is known that stable deoxycholic acid–alcohol complexes exist (20), the association between the deoxycholic acid and alcohol at these high alcoholic concentrations must be much greater than that for the reserpine–deoxycholic acid interaction. This and the decreased solubility of reserpine in high alcoholic concentrations could account in part for the marked decrease in the observed interaction between reserpine and deoxycholic acid.

The plateau region obtained in the 40% plot found in Fig. 11, representing the solubility limit of deoxycholic acid at this alcoholic strength, made possible the calculation of the apparent stoichiometry that was found to be 1:1. The multislope isotherms observed for the interaction in these alcohol–water systems (*e.g.*, 50%) could represent the successive formation of a series of higher order complexes in solution and certainly illustrate the complexity of this interaction. This was also mentioned as a possible explanation for similar multislope systems observed in many of the sodium deoxycholate interactions, although in these systems, micelle formation is also a definite possibility. It is interesting to note that the stoichiometries determined from the isolation and subsequent analysis of deoxycholic acid complexes prepared from saturated alcoholic solutions are usually of higher order (6:1, 8:1, etc.), while stoichiometries obtained from the solubility isotherms (1:1 for the reserpine–deoxycholic acid complex) are much lower. Such differences in the reported stoichiometries of deoxycholic acid interactions indicate that the complexes formed can be

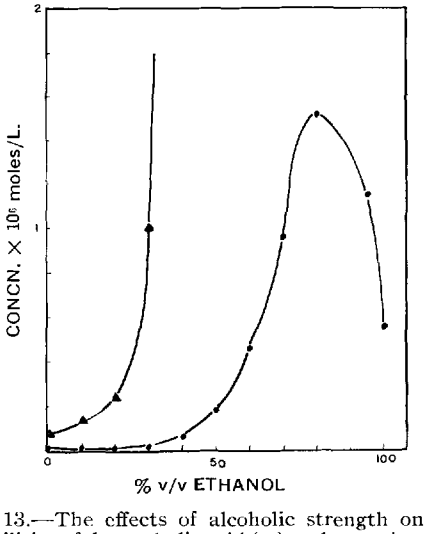

Fig. 13.—The effects of alcoholic strength on the solubilities of deoxycholic acid (▲) and reserpine (●).

greatly influenced by the type of solvent system employed (20).

CONCLUSION

Both β-cyclodextrin and sodium deoxycholate are shown to interact with a variety of pharmaceutical compounds. While varying concentrations of sodium deoxycholate would be expected to have a measurable effect on the pH of systems at lower pH levels, no effect was observed in these investigations because of the elevated pH conditions employed. In general, greater complexing activity is observed between the smaller guest molecules and both complexing agents, illustrating the importance of molecular size and structure in these interactions. Compounds known to be too large for complete inclusion, such as morphine and reserpine, are seen to interact with β-cyclodextrin. The presence of certain functional groups in these large molecules, capable of interacting with or of being partially included by the cyclodextrin, could be responsible for the observed interactions. The high-formation constants determined for some of the β-cyclodextrin interactions indicate the formation of extremely stable complexes. Pure inclusion is described as an association taking place without intermolecular bonding between the guest and host components. Although in aqueous solution, the net interactions could result from both inclusion and intermolecular forces, particularly hydrogen bonding.

Similarities in the shapes of solubility isotherms obtained for interactions of both deoxycholic acid and its sodium salt with several pharmaceutical compounds could indicate similar mechanisms for these two complexing agents. Even though sodium deoxycholate, an anionic surfactant, is currently thought to exert its solubility effects through micelle formation, the possibility of total or even partial inclusion formation by this agent cannot be ignored. Multislopes obtained in the solubility isotherms for many of the sodium deoxycholate interactions could indicate a complex mechanism consisting of both micellar solubilization and inclusion formation, or they might represent the presence of higher order complexes. The complexity of these interactions is clearly shown in the studies dealing with the reserpine–deoxycholic acid interaction in various alcoholic solutions.

REFERENCES

(1) Higuchi, T., and Lachman, L., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 521(1955).
(2) Lachman, L., Ravin, L. J., and Higuchi, T., *ibid.*, **45**, 290(1956).
(3) Lachman, L., and Higuchi, T., *ibid.*, **46**, 32(1957).
(4) Lachman, L., Guttman, D., and Higuchi, T., *ibid.*, **46**, 36(1957).
(5) Reigelman, S., *ibid.*, **49**, 339(1960).
(6) Lach, J. L., and Chin, T.-F., *J. Pharm. Sci.*, **53**, 924(1964).
(7) Schlenk, H., Sand, D. M., and Tillotson, J. A., *J. Am. Chem. Soc.*, **77**, 3587(1955).
(8) French, D., *et al.*, *ibid.*, **71**, 353(1949).
(9) Higuchi, T., and Lach, J. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 349(1954).
(10) Lach, J. L., and Cohen, J., *J. Pharm. Sci.*, **52**, 137 (1963).
(11) Thoma, J. A., and Stewart, L., "The Cycloamyloses," to be published.
(12) Cohen, J., and Lach, J. L., *J. Pharm. Sci.*, **52**, 132 (1963).
(13) Lach, J. L., and Chin, T.-F., *ibid.*, **53**, 69(1964).
(14) Higuchi, T., and Zuck, D. A., *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 138(1953).
(15) Ekwall, P., *Chem. Abstr.*, **49**, 1912(1955).
(16) Boven, R., and Ruyssen, R., *Mededel. Vlaam. Chem. Ver.*, **18**, 102(1956).
(17) Furusawa, T., *Fukuoka Igaku Zassi*, **53**, 124(1962).
(18) Lordi, N. G., *et al.*, *J. Pharm. Sci.*, **53**, 463(1964).
(19) Paruta, A. N., Sciarrone, B. J., and Lordi, N. G., *ibid.*, **54**, 838(1965).
(20) Giacomello, G., and Bianchi, E., *Gazz. Chim. Ital.*, **73**, 3(1943).

Synthesis of *N*-Substituted Phenethylamines and Corresponding Cyclohexyl Analogs

Preliminary Evaluation as Bronchodilators

By JOHN B. DATA, MARTIN O. SKIBBE, T. LAMAR KERLEY, and LAWRENCE C. WEAVER

A series of *N*-substituted phenethylamines and their corresponding cyclohexyl analogs were prepared and tested pharmacologically for their effects on the duration of hexobarbital anesthesia in mice, and systemic blood pressure and bronchodilatory activity in dogs. Methods for the preparation of these compounds are described, and procedures used in pharmacological testing are indicated and the biological results tabulated. There were no consistent or appreciable bronchodilatory effects observed.

EPINEPHRINE (I) and isoproterenol (II) represent two potent and useful bronchodilators containing the catechol nucleus. Other structurally related substances which have been rated (1–3) as relatively potent bronchodilators are levarterenol (III), 3,4-dihydroxyephedrine (IV), and equine (V). A recently introduced adrenergic substance used in the management of bronchial asthma is the potent inhibitor, protokylol (VI).

Received August 6, 1965, from the Medicinal Chemistry Department, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Ind., and the Biomedical Research Department, Pitman-Moore Division of the Dow Chemical Co., Indianapolis, Ind.
Accepted for publication October 15, 1965.
The synthetic work was supported by a grant from the American Foundation for Pharmaceutical Education.
Martin O. Skibbe expresses his appreciation to the American Foundation for Pharmaceutical Education for financial aid.