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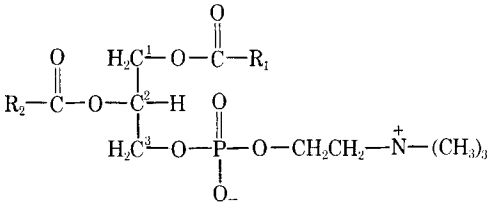
Notes

Solubilization and Rate of Dissolution of Drugs in the Presence of Physiologic Concentrations of Lysolecithin

By THEODORE R. BATES*, SONG-LING LIN, and MILO GIBALDI†

The micellar solubilizing properties of physiologic concentrations of lysolecithin for hexestrol, dienestrol, and griseofulvin are demonstrated. The extent of solubilization was found to decrease in the following order: hexestrol > dienestrol ≫ griseofulvin. Dissolution rate studies showed that lysolecithin significantly enhances the rate of solution of hexestrol, dienestrol, and griseofulvin. A role for this physiologic surfactant in the absorption process of hydrophobic drug molecules is explored.

HUMAN BILE is chiefly composed of cholesterol, calcium, bile salts, and phospholipids. Lecithin is the major phospholipid component of bile. It belongs to the class of compounds referred to as phosphatidylcholines (I).



R_1, R_2 = alkyl chains of fatty acids

I

The enzyme, phospholipase A, is capable of removing only one of the fatty acids from the lecithin molecule to form either 1- or 2-acyl lysolecithins. Phospholipase A is found in abundance in snake venom (*Crotalus adamanteus*) and also occurs in humans and animals. Its presence has been established in the mucous membrane of the small intestine (1), in duodenal fluid (2, 3), in the pancreas (4–6), and in blood serum (7). Commercially available lysolecithin is usually prepared by the enzymatic action of snake venom on purified egg lecithin. This material has palmitic or stearic acid in the 1-position of the glycerol moiety, since snake venom phospholipase A specifically catalyzes the hydrolysis of the fatty acid linkage at the 2-position (8–11).

Aqueous solutions of lysolecithin exhibit a more or less abrupt change in their physical properties over a narrow concentration range suggesting the formation of aggregates or micelles. The marked surface activity of this compound as well as other physical properties of its solutions have been reported by a number of investigators (9, 11–18). Surface tension *versus* concentration curves indicate that the critical micelle concentration (CMC) for this compound is in the concentration range of 1–2 $\times 10^{-3}$% (11, 14).

One of the most interesting properties of surface-active agents is their ability to bring into aqueous solution, at concentrations above the CMC, otherwise water-insoluble compounds. This phenomenon is known as micellar solubilization (19). There have been only a limited number of reports demonstrating the solubilizing ability of lysolecithin. Robinson and Saunders (20) found that aqueous solutions of lysolecithin possess a marked solubilizing power for cholesterol, triolein, and monostearin.

Lysolecithin appears to occur in small concentrations in human bile (21) and in high concentrations in the contents of the duodenal region of the small intestinal lumen (2). On examination of human small intestinal contents, following a test meal free of phospholipids, Borgström found that most of the phospholipids contained therein were in the form of monopalmitoyl-lysolecithin. This material was identical to that prepared by the action of snake venom on purified egg lecithin. The presence of phos-

Received June 12, 1967, from the Research Department, Ciba Pharmaceutical Co., Summit, NJ 07901, and the † Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214
Accepted for publication July 21, 1967.
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pholipase A (3) and lysolecithin (2) in duodenal fluids suggests that lysolecithin in the lumen results from the action of phospholipase on bile lecithin.

Borgström *et al.* (22) have reported that the human gall bladder is emptied during the first 30 min. after a balanced test meal, giving rise to high concentrations of bile salts and phospholipid in the content of the duodenum and proximal jejunum. The main part of the test meal is mixed with liver bile and digested and absorbed from a mixture with concentrations of bile acid of about 1 to 3 mg./ml. and of phospholipids of about 0.5 to 1 mg./ml. chiefly as lysolecithin.

The role of physiologic surface-active agents in the absorption mechanism of water-insoluble drug molecules has received scant attention. Recent reports have demonstrated the effect of physiologic bile salt solutions on the solubilization (23, 24) and dissolution rate (25) of water-insoluble drugs. On the basis of these *in vitro* findings, it was proposed that bile salts play a role in the dissolution step of the absorption process from the small intestine.

The present communication is concerned with the interaction between relatively water-insoluble drug molecules and lysolecithin, another component of the intestinal fluids, and the effect of lysolecithin on the rate of solution of these compounds.

EXPERIMENTAL

Materials—Dienestrol,[1] hexestrol,[1] and griseofulvin[2] were used as received. Chromatographically pure lysolecithin[3] was dried *in vacuo* for 24 hr. prior to use.

Equilibration—An excess quantity of drug was added to ampuls containing varying concentrations of lysolecithin solutions. The ampuls were sealed and rotated in a constant-temperature water bath at 37° until equilibrium was established. Equilibrium was determined by repetitive sampling. The solutions were then rapidly filtered through a Millipore filter assembly (0.45 μ pore size) to remove undissolved drug, and the filtrate was appropriately diluted with anhydrous reagent methanol and assayed spectrophotometrically. (See under *Assay Procedure.*)

Dissolution Rate Determination—The method employed was essentially that of Higuchi and Shefter (26). One hundred milligrams of drug in excess of its predetermined solubility in 0.05% lysolecithin solution was added to exactly 100 ml. of dissolution medium maintained at 37 ± 1° in a water-jacketed vessel. Water or a 0.05% lysolecithin solution was used as the dissolution medium.

The solution was agitated by means of an overhead stirrer (2.5-cm. blade). The agitation intensity[4] was high (800 r.p.m.) to insure complete wetting of the hydrophobic drug when water served as the dissolution medium. At appropriate time intervals a 5-ml. sample was withdrawn from the vessel and replaced by the same volume of dissolution medium. The sample was treated as outlined under *Equilibration.* A cumulative correction was made to account for the previously removed samples.

Assay Procedure—Beer's law curves were constructed for hexestrol (278 mμ), dienestrol (228 mμ), and griseofulvin (292 mμ). A methanol–water (4:1) solvent system was employed for each drug. A Beckman DB recording spectrophotometer was used to obtain absorbance readings. Lysolecithin was found not to interfere with the spectrophotometric analysis in the concentrations present in the diluted samples.

RESULTS AND DISCUSSION

The Influence of Lysolecithin Concentration on Solubilization—The solubilization curves for hexestrol and dienestrol in varying concentrations of lysolecithin at 37° are depicted in Fig. 1. It can be seen from these curves that the solubility of both drugs increases, in a linear fashion, after a certain minimum concentration (about 2.5 × 10^{-3}%) of lysolecithin has been exceeded (*i.e.*, the CMC).

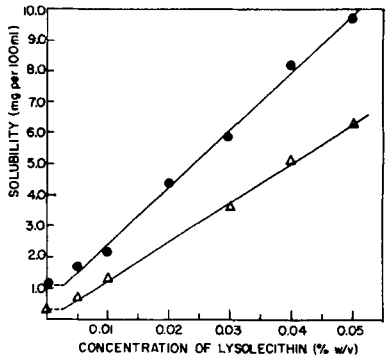

Fig. 1—Solubilization of hexestrol and dienestrol in dilute solutions of lysolecithin at 37°. Key: ○, hexestrol; △, dienestrol.

The apparent saturation capacities or ratios of the lysolecithin micelles for the drug-solubilizate molecules were obtained from the least squares slopes of the linear portion of the solubilization curves. These values, expressed as moles of micellar solubilized drug per mole of lysolecithin,[5] were found to be 0.34 and 0.23 for hexestrol and dienestrol, respectively.

Figure 2 shows the solubility of griseofulvin in solutions of lysolecithin at 37°. The saturation capacity determined for this solubilizate was 0.042.

Based on the saturation ratios found for the three solubilizates under investigation it can be seen that the lysolecithin micelle displays a somewhat similar affinity for hexestrol and dienestrol and a significantly lower affinity for griseofulvin. These differences are examples of the well established fact that the extent of micellar solubilization, in any surfactant solution, is influenced by the nature of the solubilizate molecule (19).

Lysolecithin forms essentially spherical micelles in solution, with its polar group oriented away from the

[1] Obtained from Gallard-Schlesinger Chemical Mfg. Co., New York, N. Y.

[2] Generously supplied by Schering Co., Bloomfield, N. J.

[3] Obtained from Nutritional Biochemicals Co., Cleveland, Ohio. Reported to be >99% pure by paper chromatography. Elemental analysis of the lysolecithin sample—Calcd. for monopalmitoyl-lysolecithin: C, 58.1; H, 10.2; N, 2.83; P, 6.25. Found: C, 58.4; H, 10.8; N, 2.86; P, 6.08. Elemental analysis of the fatty acid removed by heating lysolecithin in 2 *N* HCl for 48 hr. at 110° in sealed tubes—Calcd. for $C_{16}H_{32}O_2$: C, 74.9; H, 12.6. Found: C, 74.5; H, 12.7.

[4] Constant agitation intensity was achieved by utilizing a Servodyne constant torque system.

[5] Based on a theoretical molecular weight of 495.6 for monopalmitoyl-lysolecithin.

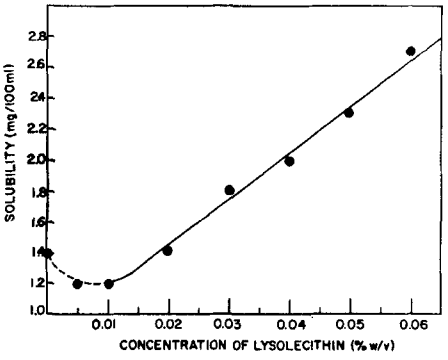

Fig. 2—Solubilization of griseofulvin in dilute solutions of lysolecithin at 37°.

"hydrocarbon" center of the micelle (14, 17, 27). Its isoelectric point is about 6.7 and in aqueous solutions it functions primarily like a nonionic surfactant (11). The lower degree of interaction of griseofulvin with the lysolecithin micelle can probably be attributed to steric effects. The volume available within the micelle for the solubilizate molecule is limited. As a result, griseofulvin being the largest of the three solubilizate molecules would encounter the greatest difficulty in packing within this restricted space.

The presence of free hydroxyl groups in the hexestrol and dienestrol molecules permits strong dipole interactions with the polar head group of the lysolecithin molecules in the micelle. This interaction may act as a driving force for the solubilization process.

Dienestrol and hexestrol are quite similar in chemical structure. The sole difference between the two molecules is the presence of double bonds in the 2 and 4 position of dienestrol, whereas the alkyl chain of hexestrol is saturated. The reduced affinity of lysolecithin for dienestrol relative to the affinity for hexestrol may be related to the fact that dienestrol is a more rigid molecule (because of conjugation) and may be restricted in its ability to pack within the micelle.

It is also of interest to compare the inverse saturation ratios (*i.e.*, moles of solubilizer/mole of drug) found with lysolecithin in the present study and with various bile salts in previous studies (23). Approximately 3 moles and 24 moles of lysolecithin were required to solubilize 1 mole of hexestrol and 1 mole of griseofulvin, respectively. The affinity of the various bile salts for hexestrol was similar to the affinity of lysolecithin for the drug. A range of about 4–6 moles of bile salt was needed to solubilize 1 mole of hexestrol. A marked difference exists, however, in the relative affinity for griseofulvin. The bile salts were much less effective solubilizers; a range of about 160–200 moles of bile salt was needed to solubilize 1 mole of griseofulvin. Nevertheless, it is apparent that both surface-active components of intestinal fluid have marked detergent properties.

The Influence of Lysolecithin on Dissolution Rate—For a drug to be absorbed across the gastrointestinal mucosa it must be in solution. In many instances the rate-limiting step in the absorption process of drugs administered in a solid form is the rate of dissolution in the gastrointestinal fluids.

Only one report has appeared in the literature concerning the influence of bile components on the dissolution rate of water-insoluble drugs (25). These investigators demonstrated that bile salts markedly enhance the dissolution characteristics of the drugs investigated.

Figures 3–5 show the dissolution behavior, in water and 0.05% lysolecithin solution, of hexestrol, dienestrol, and griseofulvin, respectively. Each curve is drawn through points which represent an average of two dissolution runs. It can be seen from these curves that the presence of lysolecithin in the dissolution medium significantly enhances the rate of dissolution of these drugs compared to the rate found in water. Inspection of these curves shows that after 2 min. the ratios of the amount of drug dissolved in the 0.05% lysolecithin solution

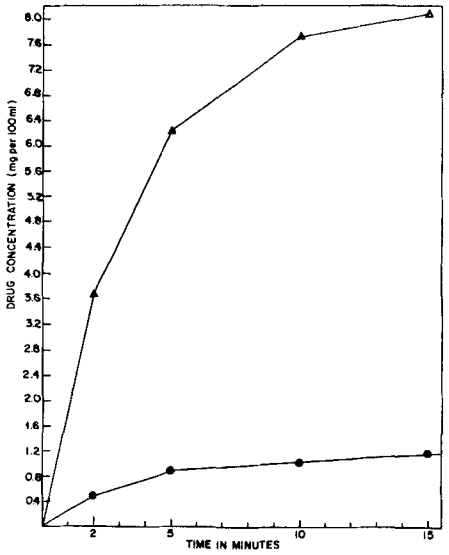

Fig. 3—Dissolution rates of hexestrol in water (●) and 0.05% lysolecithin (△) at 37°.

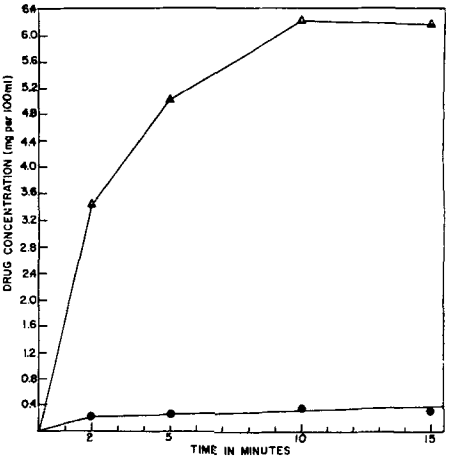

Fig. 4—Dissolution rates of dienestrol in water (●) and 0.05% lysolecithin (△) at 37°.

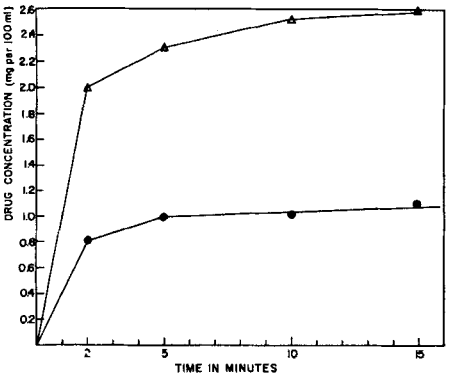

Fig. 5—Dissolution rates of griseofulvin in water (●) and 0.05% lysolecithin (△) at 37°.

to that amount dissolved in water are, 7.0, 15.6, and 2.4 for hexestrol, dienestrol, and griseofulvin, respectively. Preliminary studies indicate a threefold increase in the amount of reserpine dissolved in 0.05% lysolecithin solution at the 2-min. interval as compared to that present *at equilibrium* in water. The increased dissolution rate of these water-insoluble drugs in the presence of lysolecithin can be attributed essentially to micellar solubilization.

The presence of physiologic surfactants (*i.e.*, conjugated bile salts and lysolecithin) in the fluids of the upper region of the small intestine coupled with the strong interactions observed between these surfactants and certain poorly soluble drugs suggests that these surfactants play a role in the dissolution step of the absorption process of these pharmaceuticals.

It is anticipated that such physicochemical studies of the nature of the interactions of drugs with normal constituents of the gastrointestinal tract will significantly contribute to a greater understanding of the complex process of drug absorption.

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