Reduced Binding of Sulfaethidole to Bovine Serum Albumin in Presence of Benzalkonium Chloride

Keyphrases □ Sulfaethidole—reduced binding to bovine serum albumin in presence of benzalkonium chloride □ Benzalkonium chloride—effect on binding of sulfaethidole to bovine serum albumin □ Binding—sulfaethidole to bovine serum albumin, effect of benzalkonium chloride

To the Editor:

In an earlier publication (1) we showed that a range of acidic drugs was capable of displacing the long acting sulfonamide, sulfaethidole, from its primary binding site whereas some quaternary ammonium compounds were not. We have continued these displacement experiments with a broader range of basic drugs, using the same experimental conditions, namely 1.45×10^{-5} *M* bovine serum albumin and 2.52×10^{-5} *M* sulfaethidole in a pH 7.4 physiological phosphate buffer, for the circular dichroic technique.

The following basic drugs showed less than 2% displacement of sulfaethidole: phenylpropanolamine (7.04×10^{-4} *M*), caffeine (1.03×10^{-4} *M*), tripelennamine (3.43×10^{-5} *M*), atropine (2.88×10^{-4} *M*), propranolol (2.03×10^{-4} *M*), imipramine (2.42×10^{-5} *M*), and benzocaine (6.32×10^{-5} *M*). However, the germicide, benzalkonium[1], greatly reduced the induced ellipticity of sulfaethidole and hence its albumin binding (Fig. 1).

Benzalkonium chloride is a mixture of alkyl dimethylbenzylammonium chlorides where the alkyl chain predominantly contains 12 or 14 carbon atoms. By using an average molecular weight of 372 and assuming that one molecule of benzalkonium displaces one molecule of sulfaethidole, a binding constant of $1.4 \times 10^4\ M^{-1}$ can be calculated by the method previously described. This value of 10^4 is in good agreement with the binding constants quoted for the binding of other long chain quaternary ammonium compounds to bovine serum albumin (2–4) and suggests that benzalkonium shares at least some areas of a binding site with the anionic sulfaethidole.

The sulfaethidole curves shown in Fig. 1 do not cross over at exactly the same point and the shape of the smaller curves, those with the highest benzalkonium concentration, are somewhat distorted because the benzalkonium slightly modifies the circular dichroism of bovine serum albumin in the 250–300-nm region. This region is a fingerprint region for the aromatic residues of the protein, and the changes in the spectrum suggest a change in the polarity of the immediate environment of these residues, possibly as a result of conformational changes in the albumin.

The circular dichroism spectrum of proteins below 250 nm is primarily due to the secondary structure of the albumin; when using a benzalkonium concentration of 2.28×10^{-4} *M* and a bovine serum albumin concentration of 1.45×10^{-6} *M*, a reduction in ellipticity of 4.5% was observed in the 205–225-nm region. This finding suggests that some conformational change is produced by benzalkonium; however, the spectrum in this region is complicated by the contributions from the aromatic residues of the albumin.

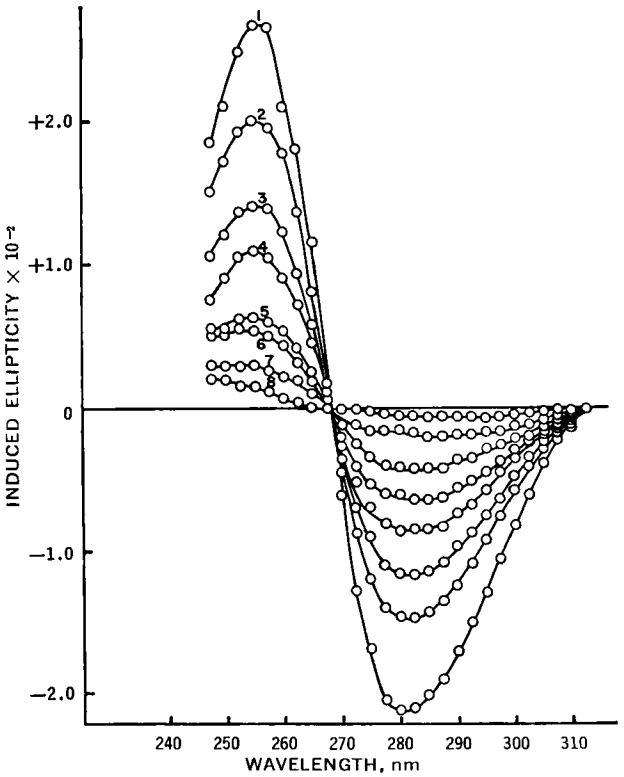

Figure 1—*Induced ellipticity of the sulfaethidole–bovine serum albumin complex in the presence of benzalkonium. Measurements were made in 10-mm cells at pH 7.4. The concentration of bovine serum albumin was 1.45 × 10⁻⁵ M, and that of sulfaethidole was 2.52 × 10⁻⁵ M. The concentrations of benzalkonium were: 1, 0; 2, 0.91 × 10⁻⁴ M; 3, 1.46 × 10⁻⁴ M; 4, 1.82 × 10⁻⁴ M; 5, 2.28 × 10⁻⁴ M; 6, 2.74 × 10⁻⁴ M; 7, 3.66 × 10⁻⁴ M; and 8, 4.57 × 10⁻⁴ M.*

If this change in ellipticity is due to conformational changes, then the changes are smaller than those found with anionic detergents under similar conditions.

Benzalkonium is usually used in a concentration of 1 in 10,000 as a preservative; even if injected, the large dilution factor would make the conformational changes in the albumin insignificant. However, the direct application of this concentration to mucous membranes may cause some denaturation of the membrane protein.

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Cholelithiasis Chemotherapy: An *In Vitro* Approach

Keyphrases □ Cholelithiasis chemotherapy—identification of cholesterol gallstone-dissolving agents, *in vitro* screening system □ Gallstones, cholesterol—dissolution, identification of drugs, *in vitro* screening system □ Cholesterol gallstones—dissolution, *in vitro* screening system to identify potential chemotherapeutic agents

To the Editor:

Cholelithiasis, a disease state resulting from deficiencies in cholesterol transport within the biliary system, is known to have afflicted humankind for centuries (1). Today, surgical removal of gallbladders and their stones is one of the most commonly performed operations with a third of a million cholecystectomies yearly in the United States alone (2, 3). Many treatments have been recommended including herbs, mineral waters, olive oil, and turpentine (4). More recent approaches to chemotherapy have generally been based on the feeding of endogenous components of bile, bile salts (5–8), or phospholipids (9). Clinical success with chenodeoxycholic acid administration was recently reported (8). Other studies have assessed the role of steroids (10), cod liver oil (11), and some drugs (12) on gallstone dissolution in rabbits fed a lithogenic diet.

The purpose of this research was to identify agents capable of bringing about the dissolution of cholesterol gallstones following oral administration. An *in vitro* screening system was developed which can monitor changes in the cholesterol-holding capacity of bile upon the addition of a test compound. The rationale for such a screening system is based on the physical–chemical properties of bile.

Bile salts will spontaneously aggregate into micelles above a critical temperature and concentration. Alone, these micelles have little ability to solubilize cholesterol; but in the presence of lecithin, the formation of mixed micelles enables the transport of large quantities of cholesterol (14). Small *et al.* (15) described this mixed micelle as a cylinder of bile salts, with hydrophilic sides facing the aqueous phase, surrounding an inner core of lecithin. The hydrophilic ends of the lecithin comprise the top and bottom of the cylindrical mixed micelle. Lecithin can interact with cholesterol to form liquid crystalline aggregates that are not soluble in aqueous media. Bile salts can engulf such aggregates to form mixed micelles and, in so doing, solubilize larger quantities of cholesterol. A complete description of these relationships may be found elsewhere (16).

The solubility of cholesterol in these four-component systems (bile salt, lecithin, cholesterol, and water) has been extensively studied. However, it has been difficult to find agreement on the same solubility values. Recently, the factors responsible for the discrepancies were identified (17) and the basal solubility of cholesterol was found to be in the order of 5 mole % (17–19). With this equilibrium solubility value defined, it has become possible to design a screening system capable of identifying compounds that can modify the equilibrium solubility of cholesterol in bile; such compounds would be potentially useful as cholelitholytic agents (20).

The cholesterol-solubilizing potential of a homologous series of quaternary nicotinic acids was studied in an aqueous *in vitro* system devoid of biliary components (13). While such a procedure measures the inherent solubilizing characteristics of a compound, it fails to identify compounds that cannot form micelles or water-soluble complexes with cholesterol but can otherwise interact with the biliary transport system to increase its carrying capacity (lecithin, for example). There is also the possibility of a compound interfering with the structure and function of the biliary micelles. Thus, it was considered worthwhile to employ a primary *in vitro* screening procedure based upon the bile salt–lecithin system. Secondary tests could then consider the inherent solubilizing ability of active compounds to gain information on their mechanism of action. A secondary test evaluating the effect of an active compound on the kinetics of gallstone dissolution is warranted; recent studies have discovered barriers to cholesterol dissolution in undersaturated bile systems (21). The development of a primary screen and some preliminary solubility enhancement data are the subjects of this report.

Excess hydrated crystalline cholesterol spiked with cholesterol-4-^{14}C was added to a lecithin–bile salt solution containing 38 m*M* egg lecithin and 135 m*M* conjugated bovine bile salts in a 0.05 *M* phosphate buffer (pH 7.4), as previously described (22). The solubility of cholesterol at 37° in this milieu was followed until equilibrium was reached. Because the long incubation times and numerous samplings of the bulk cholesterol–lecithin–bile salt suspension permitted microbial alterations, it was necessary to develop methods for sterilization and storage of the system. An incubating–stirring apparatus was designed that allowed for storage and aseptic sampling of this suspension (23).

When the cholesterol reached its equilibrium solubility, homogeneous aliquots of the suspension were placed into prewarmed vials containing the carefully weighed drug candidates. The systems were incubated with shaking at 37° for 24 hr. The solubility of cholesterol was then measured by liquid scintillation counting of an aliquot of the filtrate obtained by membrane filtration. Efficient determination of cholesterol solubility and recording of data were abetted through the use of automated liquid scintillation counting instruments equipped with punch-tape outputs. The tape was fed into a time-share terminal and, with the use of appropriate programs, back-