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Mesophase Formation during Cholesterol Dissolution in Ursodeoxycholate–Lecithin Solutions: New Mechanism for Gallstone Dissolution in Humans

Keyphrases □ Cholesterol—mesophase formation during dissolution in ursodeoxycholate–lecithin solutions, mechanism for gallstone dissolution in humans □ Gallstones—mechanism for dissolution in humans, mesophase formation during cholesterol dissolution in ursodeoxycholate–lecithin solutions

To the Editor:

It is well established that oral administration of chenodeoxycholic acid (I) induces bile desaturation in cholesterol and gradual dissolution of cholesterol gallstones in humans (1). Makino *et al.* (2) reported that ursodeoxycholic acid (II), the 7β-hydroxy epimer of I, also can induce bile desaturation in humans, an observation confirmed by other investigators (3).

The cholesterol saturation of bile (percent saturation) is defined as $(C_{sample}/C_s) \times 100$, where C_s is the concentration in the sample if it is saturated fully with cholesterol, *i.e.*, the solubility at equilibrium. In model systems, C_s is determined almost entirely by the molar proportions of bile acids and lecithin (4–6). The C_s value usually is assumed to be that value determined experimentally for the model system having an identical proportion of bile acids and lecithin, and the actual value for percentage saturation is determined graphically (7) or analytically (8). This approach has been assumed to be correct since C_s determined in bile samples from gallstone patients did not differ significantly from that of the model system (6). Accordingly, there has been rather satisfactory agreement between predicted and measured percent saturation in bile samples obtained from gallstone patients (9). The C_s value was considered to be uninfluenced by biliary bile acid composition since changing the relative proportion of the common bile acids (cholic, deoxycholic, and I) in model systems had little influence on C_s (4, 5).

Igimi *et al.* (10) reported that the ability of II to solubilize cholesterol both in the presence and absence of lecithin was strikingly inferior to that of I and concluded that II might well be less efficacious than I for gallstone dissolution. Carey and coworkers (11) confirmed these observations and also reported (12) that the maximum capacity of model bile systems to solubilize cholesterol was depressed in proportion to the percent of II conjugates in bile salt mixtures. Thus, the micellar zone in the phase diagram simulating bile appears to be reduced by the presence of II. Carey and Ko (12) also proposed that a new C_s value must be used to calculate the percent saturation in gallstone patients receiving II and presented an "urso-correction factor" to facilitate such calculations. Nevertheless, initial clinical studies (3) suggested that gallstone dissolution in patients receiving II did not occur more slowly than in those receiving I. Indeed, several instances of rather rapid dissolution were observed. To rationalize these discrepancies, we undertook a systematic study of cholesterol dissolution with II–lecithin solutions.

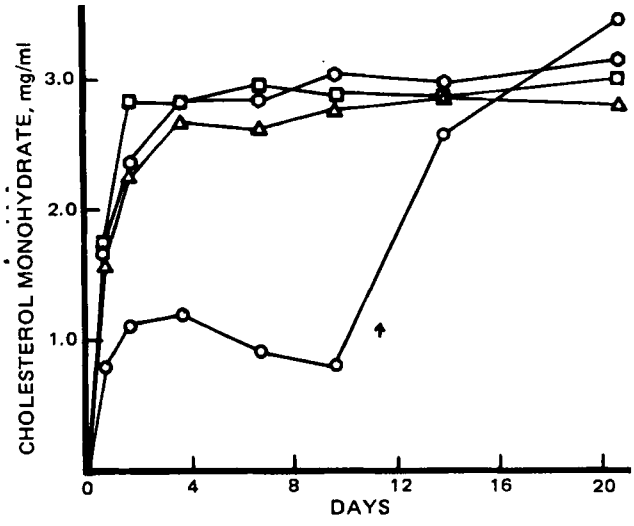

Figure 1—*Apparent solubility profiles of cholesterol monohydrate in 116 mM bile salt, 32 mM lecithin, 0.1 M NaCl, and 0.01 M phosphate buffer media at 37°. Key: ○, glycochenodeoxycholate; □, taurochenodeoxycholate; △, taurocholate; and ○, glycoursodeoxycholate; the arrow indicates the time of occurrence of a nonfilterable cloudiness due to mesophase formation in the glycoursodeoxycholate system.*

In recent dissolution studies[1] under sink conditions using methodology outlined previously (13), we found that over a wide range of bile acid, lecithin, and electrolyte concentrations, the initial dissolution rate of cholesterol monohydrate in II conjugate–lecithin media was two to 70 times lower than in corresponding I media. The dissolution of cholesterol and of gallstones *in vitro* was shown previously (9, 14) to be controlled interfacially, with the interfacial resistance $R = (h/D + 1/P)$ being a function of the dissolution medium composition. The evaluation of R and hence P (the effective permeability coefficient of the interfacial barrier) under sink conditions requires an estimate of C_s, the solute saturation concentration, since $R = AC_s/J$, where A is the area of the dissolving surface and J is the initial dissolution rate (15).

However, in contrast to media containing I and lecithin or I conjugates and lecithin, attempts to measure C_s with

[1] O. I. Corrigan, C. C. Su, W. I. Higuchi, and A. F. Hofmann, manuscript in preparation.

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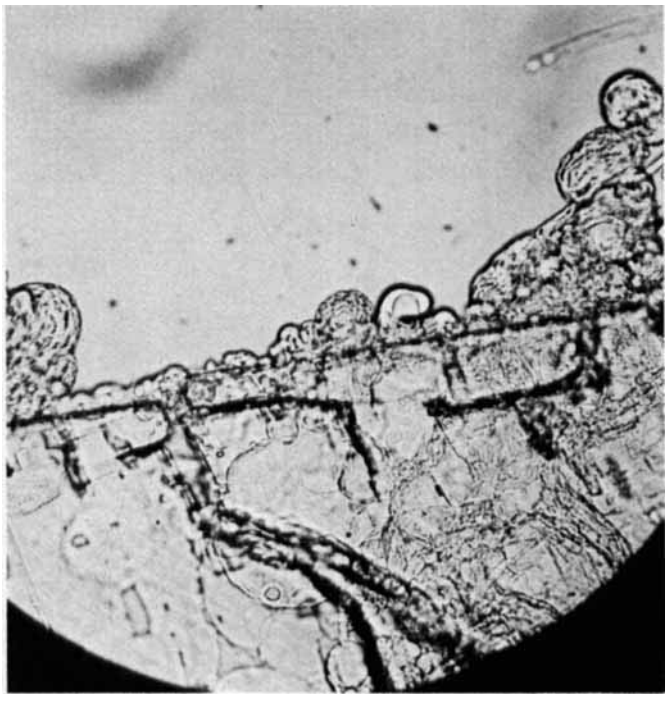

Figure 2—*Mesophase growth on cholesterol monohydrate crystal (original magnification 40×) immersed in a 174 mM ursodeoxycholate–lecithin (48 mM) medium at 37° for 20 hr.*

powder samples of cholesterol monohydrate in media containing II conjugates (glycine or taurine) and lecithin, using conventional methodology, were complicated by the development of turbidity in the supernate, which was not removed by filtration. In addition, the release of cholesterol into the II conjugate–lecithin media continued and even increased beyond the apparent equilibrium solubility (Fig. 1).

Microscopic examination of the cloudy supernate revealed a globular phase of a liquid-crystalline nature, which on analysis, following centrifugation, contained substantial amounts of lecithin (16) and cholesterol. The rate of formation of this mesophase in powder slurries, as judged by the appearance of turbidity and an increase in the apparent solubility, was dependent in the case of glycoursodeoxycholate (III) on the III–lecithin ratio and the total lipid concentration. For example, using a III–lecithin ratio of 3.61 and a III concentration of 116 m*M*, cloudiness did not occur until about the 10th day. However, at a ratio of 3.61 with a III concentration of 174 m*M* or at a ratio of 2.72 with a III concentration of 87 m*M*, the time for mesophase initiation was much shorter (~24 hr). Mesophase formation also was retarded greatly when compressed disks of cholesterol monohydrate were used rather than powder slurries.

These data and other studies[1] suggest that III has a much greater propensity than glycochenodeoxycholate (IV) to promote mesophase formation. To test this possibility, single crystals of cholesterol monohydrate were mounted as described previously (17) in a 1-cm spectroscopic cell and examined during dissolution under static conditions using a horizontally mounted microscope incubated at 37°. During dissolution in a 174 m*M* III–lecithin (48 m*M*) medium, mesophase growth on the cholesterol crystal surface became evident in a few hours. By 24 hr, the crystal surface was coated with mesophase spherulites (~40–150 μm in diameter) (Fig. 2). By contrast, crystals subjected to a IV–lecithin medium of similar composition did not show evidence of mesophase growth; dissolution was more rapid than in III.

In gallstone formation, the problem usually was considered in terms of a solid, crystalline cholesterol monohydrate phase separating from a supersaturated, isotropic micellar phase; for dissolution, the problem was thought to be the slow dissolution of crystalline cholesterol into an unsaturated micellar solution. Although a mesomorphic liquid crystalline phase occasionally has been observed in human bile samples (18, 19) or in animals in which supersaturated bile was induced (20), a mesomorphic phase generally was not considered to be involved in gallstone formation or dissolution since the mesomorphic phase occurred in model systems only at lower ratios of bile acids to lecithin than had been reported for bile samples from gallstone patients (21).

These observations indicate that mesophase formation and dispersion may occur during gallstone dissolution in patients receiving II. Since the mesophase density differs from bile, gallstone dissolution may involve flotation of a mesophase in addition to simple molecular and micellar diffusion and convection.

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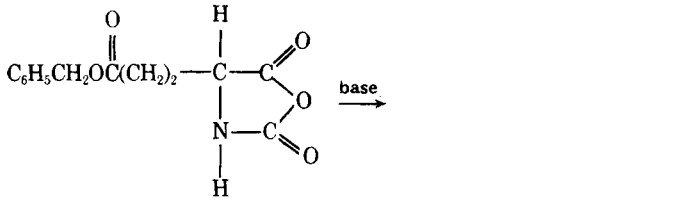
Coupling of Steroid Hormones to Biodegradable Poly(α-amino acids) I: Norethindrone Coupled to Poly-N^5-(3-hydroxypropyl)-L-glutamine

Keyphrases □ Norethindrone—coupling to poly(α-amino acids), controlled release from coupled compounds □ Contraceptives—norethindrone, coupling to poly(α-amino acids), controlled release from coupled compounds □ Polymers, biodegradable—poly(α-amino acids), coupling to norethindrone, controlled release of norethindrone from coupled compounds

To the Editor:

Sustained release of drugs from polymeric matrixes has received increased interest (1–5). Hormone release from such systems was investigated to obtain devices for long-term contraceptive purposes. A new approach in the release of hormones from polymeric systems is the use of covalently bound polymer–hormone compounds that are biodegradable and do not need removal after implantation. Although a few examples of steroids being bonded covalently to polymers have been reported (6–8), the coupling of steroids to biodegradable polymers was presented only recently (9). We wish to report a new derivatization of 17α-ethynyl-17β-hydroxy steroids, which are appropriate for attachment to biodegradable poly(α-amino acids).



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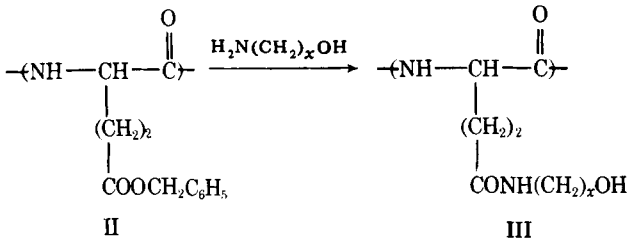


II III

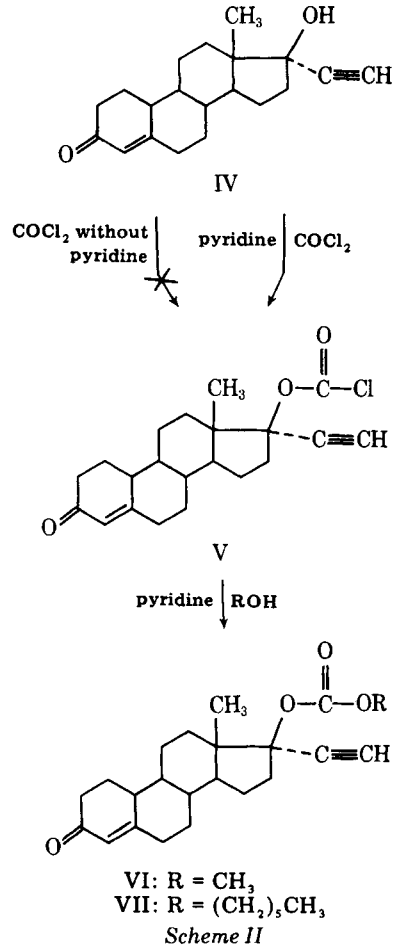
IIIa: $x = 3$

Scheme I

Poly(hydroxyalkyl)-L-glutamines (III) were studied by several investigators (10, 11). These polymers are prepared through the base-catalyzed polymerization of γ-benzyl-L-glutamate N-carboxyanhydride (I), giving the polymeric benzyl-L-glutamate (II), followed by displacement of the benzyl group with the desired hydroxyalkyl amines (12) (Scheme I). The polymers are water soluble but become water insoluble upon substitution with hydrophobic steroids.

The steroid selected for derivatization was norethindrone (IV), an active progestin (Scheme II). Reaction of IV in methylene chloride with a stoichiometric amount of pyridine and an excess of phosgene at room temperature resulted in a 77% yield of norethindrone-17β-chloroformate (V, mp 103–105° dec.). The reaction was monitored by TLC until IV had disappeared completely. The structure of V then was verified; IR (ν, KBr pellet): 1780 (chloroformate) cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$): 5.80 (s, 1H, 4[1]), 2.77 (s, 1H, 21), and 1.00 (s, 3H, 18).

Compound V is unstable and was derivatized to further confirm its structure and to investigate its potential for the coupling with polymer IIIa. Compound V was reacted with



IV V

VI: R = CH_3
VII: R = $(CH_2)_5CH_3$

Scheme II

[1] These numbers correspond to the standard nomenclature for carbon atoms of the steroid skeleton to which the protons are attached. The carbonate carbon is number 22, and the alkyl groups of the carbonate esters are numbered from 23 to 28, where 28 is the terminal carbon of the hexylcarbonate (VII).

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