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Cholesterol Dissolution Rate in Micellar Bile Acid Solutions: Retarding Effect of Added Lecithin

Abstract. *In vitro studies on the dissolution rate of cholesterol monohydrate crystals in micellar bile acid solutions showed that the addition of lecithin decreases the dissolution rate even though lecithin increases the equilibrium solubility of cholesterol in these solutions. The reduction in rates caused by lecithin was attributed to a large crystal-solution interfacial barrier. An effective permeability coefficient for the interfacial barrier was calculated to be around* 1.5×10^{-5} *centimeter per second for the transport of cholesterol molecules.*

It has been generally assumed that the solubilizing capacity of bile for cholesterol is critically related to dissolution of cholesterol gallstones in vivo (*1*). However, solubility and dissolution kinetics both appear to be important (*2, 3*), as both may be limiting factors in in vivo dissolution of cholesterol gallstones. Danzinger *et al.* (*2*) have recently reported that ingestion of chenodeoxycholic acid causes gallstone dissolution in some patients. These investigations showed that, in women with cholelithiasis, chenodeoxycholic acid causes bile to become unsaturated with respect to cholesterol. They suggested, however, that stone dissolution might be limited by the kinetics of dissolution, since duodenal bile samples obtained after gallbladder contraction were significantly undersaturated with respect to cholesterol.

A great deal of research over the past few years has emphasized the role of lecithin in increasing cholesterol solubility in bile mediums (*1, 3*). Yet little is known of the influence of lecithin on the kinetics of cholesterol dissolution.

The purpose of this report is to present in vitro findings that show the existence of an important rate-determining interfacial barrier for dissolution of cholesterol monohydrate in bile acid–lecithin mediums. Lecithin shows a strong inhibitory effect on the rate of cholesterol dissolution in cholate or taurocholate mediums. This effect was quantitatively evaluated in terms of an effective permeability coefficient of around 1.5×10^{-5} cm sec^{-1} for the interfacial barrier.

The primary process of dissolution involves the disengagement and transport of molecules from the crystal surface into the bulk solution. Equation 1 was derived to express the rate of dissolution J as a function of the diffusion coefficient D, the solubility C_s, the bulk concentration C_b, and the surface area A for a solid, when both an interfacial barrier with an effective permeability coefficient P and a Nernst diffusion layer (*4*) of thickness h are important (*5*).

$$J = \frac{A(C_s - C_b)}{h/D + 1/P} \quad (1)$$

Either of the transport barriers may be rate-determining for the process. When the interfacial resistance $1/P$ is negligible compared to the diffusional resistance h/D, the dissolution rate is dif-

Table 1. Dissolution rates J of the solute measured in solvent mediums at 37°C, with solubilities C_s and diffusion coefficients D independently determined (*6*)*, and values for h/D and $(h/D + 1/P)$ calculated according to Eq. 1.

Solute	Solvent medium	$J/A \times 10^{10}$ (mole cm^{-2} sec^{-1})	$C_s \times 10^6$ (mole ml^{-1})	$D \times 10^6$ (cm^2 sec^{-1})	$(h/D)10^{-3}$ (sec cm^{-1})	$(h/D + 1/P)10^{-3}$ (sec cm^{-1})
Cholesterol monohydrate	0.0371*M* taurocholate (*p*H 7.4)	0.43	0.30	2.15*	2.33	7.0
Cholesterol monohydrate	0.0371*M* taurocholate+ 0.0133*M* lecithin (*p*H 7.4)	0.185	1.26	1.24	4.03	67.7
Cholesterol monohydrate	0.0464*M* cholate (*p*H 8.0)	1.66	1.31	2.17	2.30	7.8
Cholesterol monohydrate	0.0464*M* cholate+ 0.0133*M* lecithin (*p*H 8.0)	0.43	2.60	1.49	3.36	60.4
Cholesterol monohydrate	0.116*M* cholate (*p*H 8.0)	4.82	3.31	1.90	2.63	6.8
Benzoic acid	0.01*N* HCl	1072.0	38.5	14.0	0.36	0.36

* Diffusion coefficient of 2.0×10^{-6} cm^2 sec^{-1} for 20 to 100 m*M* taurocholate loaded with cholesterol was reported by F. P. Woodford (*15*).

fusion-controlled, and this is the situation usually applicable to most systems. When $1/P$ is much greater than h/D, the dissolution rate is interfacially controlled. In order to establish the inhibitory effect of lecithin upon cholesterol monohydrate dissolution in cholate or taurocholate solutions, the dissolution rates, diffusion coefficients, and solubilities of cholesterol were determined independently in the mediums (*6*) (see Table 1). By Eq. 1, values for $(h/D + 1/P)$ were calculated by use of the measured J/A, C_s, and D. The magnitude of h was determined from the benzoic acid dissolution rate in 0.01*N* HCl, for which the rate was established to be diffusion-controlled (*7*). As shown in Table 1, $(h/D + 1/P)$ values about three times larger than h/D were found (*8*) for the dissolution of cholesterol monohydrate in taurocholate and cholate solutions not containing lecithin. However, when lecithin was present, values for $(h/D + 1/P)$ that were almost 18 times (*9*) larger than those for h/D were found. These results are in accord with an effective permeability coefficient P for the cholesterol interfacial transport of around 1.5×10^{-5} cm sec^{-1}. Lecithin effects of about the same magnitude have been observed for the dissolution of human gallstones in bile acid–lecithin mediums (*10*).

A similar lecithin effect has been observed in studies (*11*) of the transport of cholesterol from aqueous bile acid–lecithin solutions into hexadecane. It was found that the sterol transport is interfacially controlled, and the presence of lecithin markedly reduced the interfacial permeability coefficient. Oil-to-aqueous permeability coefficient values of around 10^{-7} cm sec^{-1} were found with taurocholate-lecithin mediums of the same composition as those shown in Table 1. Considering the fact that the area term in Eq. 1 is not the true area or microscopic surface area for cholesterol crystal dissolution, the real difference between the crystal-solution transport rates and the oil-aqueous transport rates of sterols should be somewhat smaller (*12*).

As demonstrated in this report, large interfacial barriers may be present during the dissolution of cholesterol monohydrate crystals in lecithin–bile acid solutions (*13*). These barriers are of such magnitude that they may be important in dissolution of cholesterol gallstones in vivo. Thus, the presence of such barriers may explain why the rates of gallstone dissolution in vivo observed by Danzinger *et al.* (*2*) are lower than those predicted by diffusion-controlled dissolution models (*14*), and the counteracting or the elimination of these barriers by appropriate measures may constitute a clinically significant goal.

WILLIAM I. HIGUCHI
SOMPOL PRAKONGPAN
VIJAY SURPURIYA, FUDAH YOUNG
College of Pharmacy, University of Michigan, Ann Arbor 48104

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Gas Exchange in Dry Seeds: Circadian Rhythmicity in the Absence of DNA Replication, Transcription, and Translation

Abstract. *A 24-hour rhythm of gas exchange has been detected in dry onion seeds. The rhythm persists in constant conditions, and its period appears to be independent of temperature. Since DNA replication, transcription, and perhaps translation do not occur in this quiescent state, it is concluded that the basic oscillation that defines circadian rhythmicity does not derive directly from these processes.*

Perhaps the most frustrating aspect of work with circadian rhythms is our lack of knowledge concerning the nature of the basic oscillation that ultimately determines the period of the fluctuation. In recent years attention has been focused on transcriptional and translational processes as the site of the basic oscillator (*1*). This line of reasoning has culminated in the "Chronon" concept (*2*), which postulates that the genome has evolved un-