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Participation of Micelle at Crystal–Solution Interface in Rate-Determining Step for Cholesterol Gallstone Dissolution in Unsaturated Bile Media

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Keyphrases ☐ Cholesterol gallstone dissolution rate in unsaturated bile media—effect of micelle at crystal–solution interface ☐ Gallstone dissolution rate in unsaturated bile media—effect of micelle at crystal–solution interface ☐ Micellar effect—cholesterol gallstone dissolution rate in unsaturated bile media ☐ Bile salt–lecithin micelle—effect on transport of cholesterol in interface, effect on gallstone dissolution rate

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Sir:

Recent investigations (1–3) in this laboratory have established that the *in vitro* dissolution of human cholesterol gallstones in bile salt–lecithin media is largely controlled by crystal–solution interfacial kinetics rather than by bulk solution diffusion. Apparent interfacial barriers for dissolution were determined (2, 3) for a variety of cholesterol stones; these were typically 15–20 times greater than bulk diffusion barriers in the *in vitro* experiments (2, 3). Similar dissolution rate experiments with compressed pellets of cholesterol monohydrate crystals have yielded comparable results—*viz.*, interfacial barriers 20 times greater than bulk diffusion in sodium taurocholate–lecithin and sodium cholate–lecithin solutions (2, 3). These results have encouraged the mechanistic exploration of the rate-determining step(s) at the cholesterol monohydrate crystal–solution interface.

This communication reports data showing that the bile salt–lecithin micelle is critically involved in the "activated complex" for the transport of cholesterol in the interfacial region. Table I gives the results of a dissolution rate study showing the relative constancy of the effective interfacial transport coefficient, P, when the bile salt–lecithin concentration is changed while the bile salt–lecithin ratio is kept constant. As can be seen,

Table I—Dissolution Rate[a] of Cholesterol Monohydrate Pellets Showing the Direct Dependence of the Rate, J/A, upon the Solubility, C_t, and the Relative Independence of the Rate upon the Interface Transport Coefficient, P

Sodium Cholate + Lecithin, % (in 0.1 M Phosphate, pH 7.4)	(J/A)[b]10^4, mg./cm.^{2}/ sec.	C_t[c], mg./cm.^{3}	$D^d \times 10^{6*}$, cm.^{2}/sec.	$P^f \times 10^6$, cm./sec.
1 + 0.5	0.057	0.30	1.46	1.90
2 + 1.0	0.16	1.05	1.49	1.52
2 + 1.0 + 0.07 M NaCl	0.14	1.00	1.49	1.40
ionic strength (correct to 5% cholate)				
5 + 2.5	0.56	3.00	1.30	1.87

[a] Experimental methods for determining J/A, C_t, and D are given in Reference 3. [b] $J/A = PC_t$ = dissolution rate (mg./cm.^{2}/sec.). [c] C_t = total solubility of cholesterol monohydrate. [d] D = effective diffusivity of cholesterol in micellar solution. [e] Diffusion coefficient data are presented mainly to show that the size of the micelle is not changing greatly over the concentration range studied. [f] P = interface transport coefficient (cm./sec.).

P remained essentially constant when the solubility, C_t, varied by about a factor of 10. Since it was already demonstrated (2, 3) that the process is interface-kinetics controlled, these data can only be interpreted by some mechanism in which the bile salt–lecithin–cholesterol micelle is involved in the rate-determining step.

This finding is exciting for a number of reasons. The recent studies of Surpuriya and Higuchi (4, 5) showed that the oil–water transfer of a variety of sterols in several bile salt–lecithin systems is also interfacial-barrier controlled and that the bile salt–lecithin–cholesterol micelle is involved in the rate-determining step. Thus the basic mechanisms may be very similar for the two situations, and other parallel characteristics are anticipated. For example, Surpuriya[1] showed that the presence of 0.01 M calcium chloride increases P by a factor of 5 in his oil–water system. It would indeed be interesting to see similar effects in the gallstone and/or cholesterol monohydrate dissolution.

There is a significant clinical aspect with regard to the present findings. Recently, Danzinger *et al.* (6) showed that oral administration of chenodeoxycholic acid to patients suffering from gallstone disease can lead to *in vivo* dissolution of gallstones. In four of the seven patients studied, these investigators found that gallstones either disappeared completely or diminished significantly in size during the 14–22 months of chenodeoxycholic acid treatment. In the remaining three patients, no changes in gallstone size could be determined. These clinical studies support the idea that increasing the *in vivo* dissolution rate by a factor of 10 or more should yield material patient benefits in that practical medical treatment times may be approached.

The present findings and the parallel work on the oil–water transport of sterols could be of value in seeking out agents, additives, and other biopharmaceutical factors important to the safe and efficacious treatment of gallstone sufferers.

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Figure 1—*Degradation of nitroglycerin as followed by absorbance change at 336 nm.; [nitroglycerin]* ≃ *0.4 mg./3 ml. of reaction mixture; [sodium hydroxide]* = *0.03* M; *solvent* = *99% methanol; 25°.*

Kinetic Assay of Single Nitroglycerin Tablets

Sir:

Current interest in the stability of nitroglycerin tablets (1, 2) demonstrates the need for a simple, rapid, and precise single-tablet assay procedure for nitroglycerin. The present USP method (3) requires separation of the drug from potentially interfering substances by column chromatography followed by acid hydrolysis, which yields the nitrate ion, and subsequent spectrophotometric determination of nitrated phenoldisulfonic acid. Its complexity of manipulation does not permit this method to be rapid and efficient for the determination of nitroglycerin in single tablets. The GC (4) and polarographic (5) methods are accurate and specific but require extensive instrumentation. Simpler methods, such as that developed by Bell (6), are nonspecific; they can be subject to interference either by nitrate or nitrite ions.

The recent accelerated research activities in nitroglycerin formulations prompted us to report our preliminary data on a kinetic assay of nitroglycerin in tablets. This assay method is simple, rapid, and precise and appears to be superior to existing methods for the single-tablet assay of nitroglycerin.

Nitroglycerin has no significant UV absorption above 240 nm.; but in alkaline solutions, it degrades *via* a chromophoric intermediate which exhibits an absorption peak around 336 nm. For example, in 99% methanol and at a sodium hydroxide concentration of 0.03 *M*, the appearance and disappearance of peak intensity at 336 nm. (Fig. 1) can be ascribed to $A \rightarrow B \rightarrow C$ kinetics. The kinetic order, rate of degradation, and maximum peak absorbance are dependent on nitroglycerin and sodium hydroxide concentrations and solvent composition. A detailed description of the kinetics and mechanism of nitroglycerin degradation in
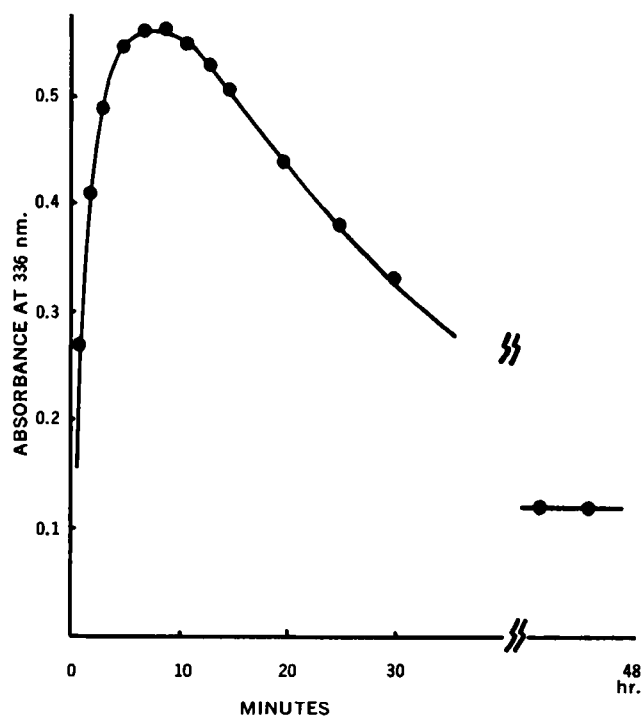
alcoholic sodium hydroxide solutions will be presented elsewhere[1].

At a constant sodium hydroxide concentration and solvent composition, the observed maximum absorbance at 336 nm. was found to be a linear function of nitroglycerin concentration. A convenient system was 33% water in methanol and 0.033 *M* NaOH in the reaction mixture. The kinetic assay could be initiated either by placing 1 ml. of aqueous nitroglycerin solution or dissolving the sublingual tablet with 1 ml. of water directly in a 1-cm. cell, followed by addition of 0.05 *M* NaOH in methanol. The absorbance at 336 nm. was then followed until a maximum value was reached.

The validity of the present assay technique was checked against the official USP method. Different quantities of nitroglycerin tablets[2] and powders[3] were dissolved in water, and the solutions were assayed simultaneously by the USP and the kinetic methods. In the USP assay, 1-ml. aliquots of nitroglycerin solutions were mixed with the indicated amount of column material[4] and applied to the column. The same volume of nitroglycerin solution was used in the kinetic assay. Figure 2 shows a plot of absorbance maximum at 336 nm. as a function of the concentrations of nitroglycerin in the prepared solutions as determined by the USP method. The excellent linearity observed (correlation coefficient = 0.994 for 14 points) strongly testifies to the validity of the kinetic assay.

The slight positive intercept observed in Fig. 2 was apparently contributed by the tablet diluent (lactose)

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[3] One part of nitroglycerin plus nine parts of lactose USP, supplied by ICI America Inc., Atlas Chemicals Division, Wilmington, DE 19899
[4] Celite 545, Fisher Scientific Co., Fair Lawn, N. J.