The thyroxine iodine level was determined at the onset of the 6-day experiment and again 36 hr after discontinuance of the drug, when only a 4% difference in thyroxine iodine level was noted (7.9 μg/100 ml initially *versus* 8.2 μg/100 ml after drug discontinuation); this difference is considered within the normal range.

DISCUSSION

Organically bound iodine as iodinated glycerol has been widely used as a mucolytic expectorant for over 50 years. It has been reported to be as effective an expectorant as a saturated solution of potassium iodide and to have fewer side effects (3–5), the latter being attributed to the smaller amounts of iodine needed.

Gastric distress is frequently encountered with the use of a saturated solution of potassium iodide and, less commonly, iodism as evidenced by skin rash. Thyroid enlargement is not uncommon with longrange therapy with this drug. On the other hand, Seltzer (3) reported that in 100 consecutive cases treated with iodinated glycerol, there was no instance of thyroid enlargement, iodism, or complaints of gastric irritation. In 100 cases treated with a saturated solution of potassium iodide, he found four cases of iodism and three of thyroid enlargement.

It is self-evident that one must seek that expectorant activity of iodine that can be accomplished with the lowest possible dose and the least side effects, such as can be brought about with organically bound iodine formulations, as long as the formulation is bioavailable. This study has demonstrated that three such formulations containing organically bound iodine are not only bioavailable but are also approximately equiavailable in relation to time and dose.

CONCLUSIONS

The bioavailability of orally administered organically bound iodine as iodinated glycerol was demonstrated in adult, human, healthy subjects.

Total blood iodine levels following single recommended therapeutic doses of iodinated glycerol showed a rapid rise and a progressive fall over the experimental 24-hr period, at which point there was still an elevated level above initial concentrations. The thyroxine iodine level was not significantly affected after such single doses.

When daily doses of iodinated glycerol were continued for 6 days, it was demonstrated that the total blood iodine rose rapidly, maintained a constant level, and, after discontinuation, returned progressively within 48 hr to preadministration levels. The thyroxine iodine level remained within the normal range after continued medication over this period.

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Enhancing Effect of Calcium Ions on Transport of Cholesterol from Aqueous Sodium Taurocholate–Lecithin Micellar Phase to Oil Phase

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Abstract □ Experiments on the influence of calcium ions upon the transport of cholesterol from bile salt–lecithin micelles into an oil phase were carried out using previously developed methods. As the calcium-ion concentration is increased, the sterol transport rate increases until a limiting maximum rate is reached at around a 0.03 *M* concentration of calcium ions. This limiting rate corresponds to an interfacial barrier permeability coefficient, *P*, of about 1×10^{-6} cm/sec, which is 35–40 times larger than that obtained in the absence of calcium. These results are consistent with the interfacial barrier-controlled mechanism in which the bile salt–lecithin micelle is involved in the rate-determining step at the aqueous–lipid interface.

Keyphrases □ Calcium ions—enhancing effect on cholesterol transport from sodium taurocholate–lecithin micelles to oil phase □ Cholesterol transport—from bile salt–lecithin micelles into oil phase, enhancing effect of calcium ions □ Bile salt–lecithin micelles—transport of cholesterol, influence of calcium ions

Recent investigations (1–6) in these laboratories led to the conclusion that the transport of cholesterol and other sterols from aqueous to oil phases may be interfacial barrier controlled rather than bulk phase diffusion controlled. The implications of these findings in biology should be broad, because the understanding of sterol transport is vital to the understanding of sterol absorption in the intestine, of the pathogenesis of atherosclerosis and of cholesterol gallstones, and of the metabolism of sterols generally. That interfacial kinetics rather than bulk diffusion and convection are more important relegates thermodynamics into somewhat of a secondary role as far as transport is concerned. Previous studies (5, 6) showed not only that the kinetics in this situation are interfacial barrier controlled but that the bile salt–lecithin micelle is a key participant in the rate-determining step.

The present report represents a portion of the effort made to understand the mechanistic role of the bile salt–lecithin–cholesterol micelle in the transport of cholesterol at the lipid–aqueous interface. The present data demonstrate a dramatic effect of calci-

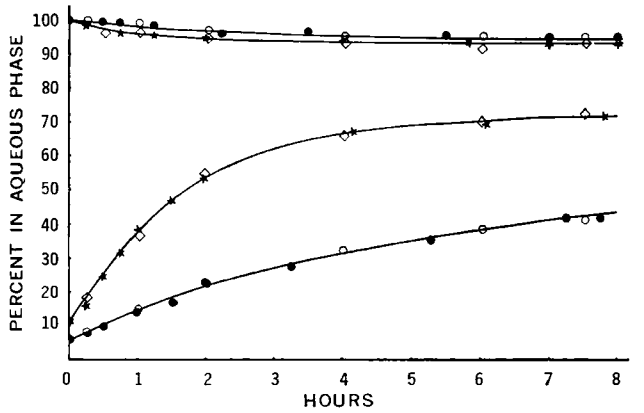

Figure 1—*Comparison of experimental data with theory for the uptake and release of cholesterol in the sodium taurocholate–lecithin system with 0.8% hexadecane. Key: ●, experimental, 2% sodium taurocholate + 1% lecithin; ★, experimental, 2% sodium taurocholate + 1% lecithin + 0.01 M calcium chloride; and ○ and ◊, duplicate experiments. Smooth curves represent theoretical predictions.*

um ions upon the cholesterol transport rate in the context of an interfacial barrier-controlled situation and, therefore, provide a significant clue toward the determination of the nature of the "activated complex" and of the various factors contributing to the interfacial transfer process.

EXPERIMENTAL

The same general procedures utilized previously (5, 6) were followed. Basically, the experiments involved the preparation of master emulsions with hexadecane[1] and appropriate sodium taurocholate[2]–lecithin[2] solutions by homogenizing in a blender[3], shaking for about 15–20 min, and then diluting in the sodium taurocholate–lecithin solutions in which appropriate amounts of calcium chloride[4] and/or sodium chloride[4] were added beforehand. Radioactive cholesterol[5] was either added to the oil (for release experiments) or to the sodium taurocholate–lecithin solution (for uptake experiments). The mixture was then shaken gently at 30° in a wrist-action shaker[6]. Five-milliliter samples were pipeted out at different time intervals, and the aqueous supernatant phases were separated from the oil by high speed centrifugation[7] for 1–1.5 min. Out of the clear aqueous solution collected, 1 ml was pipeted into a liquid scintillation vial and 10 ml of a liquid scintillation cocktail[8] was then added to the vial. The samples were quantitatively analyzed in a liquid scintillation counter[9].

Individual emulsion particle-size distribution data[10] were obtained for each emulsion, as before. Generally, no significant changes in the particle-size distribution occurred in the reaction systems during the run, even when the maximum levels (0.1 *M*) of calcium ions were present.

RESULTS

The plots of a typical transport experiment (Fig. 1) give the percent of the radioactive compound in the aqueous phase as a function of time. As can be seen, the reproducibility of the experiments was quite satisfactory.

The experimental results were treated by the following equations, developed earlier (5), based upon the physical model shown in Fig. 2:

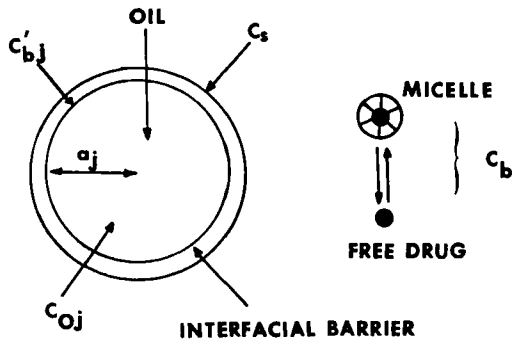

Figure 2—*Physical model describing the uptake and/or release of solutes by the oil droplet;* a_j = *radius of oil droplet,* C_{oj} = *solute concentration in oil phase,* C_s = *aqueous solute concentration just outside adsorbed film,* C_b = *solute concentration in aqueous phase, and* C'_{bj} = *solute concentration at oil–water interface.*

$$\frac{dC_{oj}}{dt} = \frac{3DP(C_b - C_{oj}/K)}{a_j(D + a_jP)} \qquad \text{(Eq. 1)}$$

$$\frac{dC_b}{dt} = -\frac{4\pi}{3V_w}\sum_{j=1}^{L} a_j^3 \Delta N_j \frac{dC_{oj}}{dt} \qquad \text{(Eq. 2)}$$

where:

a_j = radius of oil droplet
P = apparent permeability coefficient for interfacial barrier
D = relevant diffusion coefficient for cholesterol in bile salt–lecithin solution
C_b = total bulk aqueous solute concentration
C_{oj} = concentration of solute in oil droplet
K = effective hexadecane–(bile salt–lecithin) partition coefficient for solute
V_w = volume of aqueous phase
ΔN_j = number of droplets of sizes between A_j and A_{j+1}
L = largest oil droplets in system

Equation 1 expresses the rate of change in the concentration of the solute in the oil droplet, and Eq. 2 describes the material balance in the system. These two equations were solved[11] for C_b when V_w, D, P, K, and the particle-size distribution were known. Fitting of the experimental data by the theory was accomplished by using P as the only adjustable parameter. The K values were

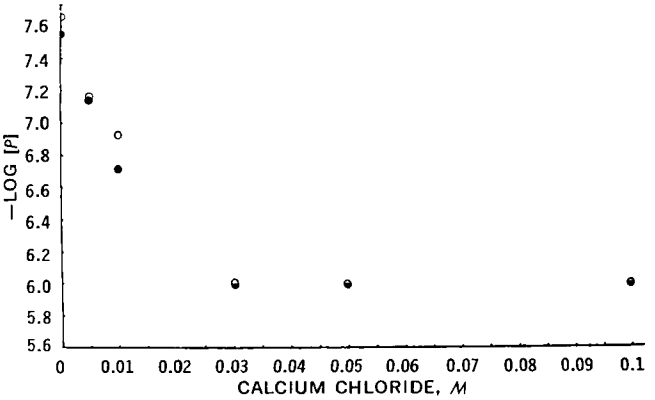

Figure 3—*Effect of increasing concentrations of calcium chloride on the permeability coefficient* (P). *Plot shows* −*log* [P] *versus molar concentration of calcium chloride. Key: ○, 1% sodium taurocholate + 0.05% lecithin; and ●, 2% sodium taurocholate + 1% lecithin.*

[1] Matheson, Coleman and Bell, Norwood, Ohio.
[2] Schwarz/Mann, Orangeburg, NY 10962
[3] Waring, Sargent Welch Scientific Co., Chicago, Ill.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Cholesterol-4-C^{14}, New England Nuclear, Boston, Mass.
[6] Burrell, Burrell Corp., Pittsburgh, Pa.
[7] Lourdes Instrument Corp., Brooklyn, N.Y.
[8] The cocktail consisted of 2,5-diphenyloxazole (7 g), 1,4-bis[2-(5-phenyloxazolyl)]benzene (50 mg), naphthalene (50 g), and dioxane *q.s.* (1000 ml).
[9] Beckman Instruments, Inc., Fullerton, Calif.
[10] Using the Coulter counter, model A, Coulter Electronics, Hialeah, Fla.
[11] Using an IBM-360 digital computer.

Table I—Effect of Increasing Amounts of Calcium Chloride and/or Sodium Chloride on the Partition Coefficient (K) and the Effective Permeability Coefficient (P) for Cholesterol in the Sodium Taurocholate–Lecithin System

2% Sodium Taurocholate + 1% Lecithin					1% Sodium Taurocholate + 0.5% Lecithin				
Electrolyte, M			$P \times 10^7$, cm/sec		Electrolyte, M			$P \times 10^7$, cm/sec	
Calcium Chloride	Sodium Chloride	K	Uptake	Release	Calcium Chloride	Sodium Chloride	K	Uptake	Release
0.00	0.00	10.0	0.30	0.28	0.00	0.00	19.4	0.22	0.21
0.005	0.00	10.0	0.71	0.70	0.005	0.00	19.0	0.69	0.68
0.01	0.00	9.0	1.9	1.8	0.01	0.00	18.0	1.20	1.20
0.03	0.00	10.0	10.0	10.0	0.03	0.00	18.0	9.80	9.70
0.05	0.00	10.0	10.0	10.0	0.05	0.00	18.0	10.0	10.0
0.1	0.00	10.0	10.0	10.0	0.1	0.00	17.0	9.8	10.0
0.00	0.1	8.0	0.40	0.40	0.00	0.1	20.0	0.32	0.32
0.005	0.1	9.0	20.0	20.0					
0.01	0.1	9.0	20.0	20.0					

calculated from the plateau portions of the uptake and release plots of the experimental data. The computer simulation calculations are shown as the smooth curves in Fig. 1.

Table I gives the K values and the best P values for cholesterol in the sodium taurocholate–lecithin system with added calcium chloride and/or sodium chloride. As can be seen, the P values obtained from the uptake experiments and those obtained from the release experiments are in close agreement.

DISCUSSION

Table I shows the dramatic effect of added calcium chloride upon the P values of cholesterol in the sodium taurocholate–lecithin system. As the concentration of calcium chloride is increased, the P values increase until a maximum of 1×10^{-6} cm/sec is reached (Fig. 3). At 0.01 M calcium chloride, a P value of about $1.2\text{–}1.8 \times 10^{-7}$ cm/sec is obtained, which is about six to seven times larger than the value obtained without calcium chloride; at 0.1 M calcium chloride, a P value of about 1×10^{-6} cm/sec is obtained, which is about 35–40 times larger than the control. Table I also shows the effect of added sodium chloride upon the P values, and that effect is nothing like that exhibited by calcium. Even at 0.1 M sodium chloride concentration, a P value is increased by only a factor of about 1.5.

Table I shows that varying the concentration of sodium taurocholate and lecithin but keeping their ratio constant does not affect P; *i.e.*, P is independent of the taurocholate–lecithin concentration and, therefore, of K. This supports the previously proposed mechanism (5, 6) implicating the micelle in the rate-determining step. The presence of lecithin in the micelle would be expected to increase the affinity of the micelle for the sterols greatly. Consequently, it is reasonable to expect that the release tendency for the sterol from the micelle should be reduced when lecithin is present in the micelle.

The aqueous to oil transport of cholesterol from sodium lauryl sulfate micelles probably follows an entirely different mechanism (5). The effect[12] of sodium chloride on the transport rates of cholesterol in the sodium lauryl sulfate was that the electrolyte significantly reduced the P values. In this case, the free solute transport mechanism (5) rather than the micelle transport mechanism is probably the rate-determining one.

The present finding that calcium ion greatly increases the permeability coefficient for cholesterol in the sodium taurocholate–lecithin–hexadecane system is very provocative. The calcium-ion effect is much larger than might be expected on the basis of simple ionic strength effects, *e.g.*, the influence of sodium chloride. Also when calcium ions and sodium ions are added together in this sodium taurocholate–lecithin system, the P value for cholesterol is increased to 2×10^{-6} cm/sec (Table I), which indicates that the effect of calcium ions and sodium ions is synergistic rather than additive and strongly supports the proposal that the effect of calcium ions may be different and more specific than sodium ions and that it can play a key role in lowering the free energy of the activated complex involved in the transport process.

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