latter may have been a result of the slightly larger dose of the 165-mg capsule. The equivalence of the capsule formulations with respect to all other variables suggests that any differences in the bioavailabilities of the three formulations are inconsequential.

According to previous reports (20, 21), fenoprofen pharmacokinetics after an oral or intravenous dose of 250 mg can be adequately represented by a two-compartment open model. Under such a model, plasma levels are a linear function of the dose administered (22).

The results of Study 1 are sufficient to corroborate linearity of orally administered fenoprofen pharmacokinetics in the 60–300-mg dose range only if nonlinearities are not obscured by bioinequivalencies of the three capsule formulations. Since Study 2 established that the three formulations are essentially bioequivalent, the differences among the formulations in Study 1 probably were due solely to differing doses. Since the Study 1 bioavailability parameters are proportional to the dose, as established by the analysis of variance of the adjusted variables, these experiments provide additional evidence that orally administered fenoprofen pharmacokinetics are linear over the 60–300-mg dose range.

A previous study (23) reported nonlinearity of plasma levels to high doses of another α-methylarylacetic acid, naproxen. The nonlinearity occurred at doses of >500 mg while lower doses two times a day yielded a linear dose–response curve. This fenoprofen study included single doses of 60–300 mg. Higher fenoprofen doses were not investigated.

REFERENCES

(1) J. A. Wojtulewski, *Curr. Med. Res. Opin.*, **2**, 551 (1974).
(2) H. S. Diamond, *J. Rheumatol., Suppl. 2*, **3**, 67 (1976).
(3) J. W. Brooke, *ibid.*, **3**, 71 (1976).
(4) F. G. McMahon, A. Jain, and A. Onel, *ibid.*, **3**, 76 (1976).
(5) E. C. Huskisson, *Curr. Med. Res. Opin.*, **2**, 545 (1974).
(6) P. M. Reynolds and P. J. Whorwell, *ibid.*, **2**, 461 (1974).
(7) R. Marcolongo, E. Camarri, M. Tocca, and E. Fanteria, *Clin. Ter.*, **75**, 449 (1975).
(8) J. Zuckner and R. J. Auclair, *J. Rheumatol., Suppl. 2*, **3**, 18 (1976).
(9) O. B. Gum, *ibid.*, **3**, 26 (1976).
(10) I. F. Duff, J. E. Neukom, and J. E. Himes, *ibid.*, **3**, 32 (1976).
(11) W. J. Blechman and S. Zane, *ibid.*, **3**, 38 (1976).
(12) S. H. Roth, D. W. Englund, and B. K. Harris, *ibid.*, **3**, 43 (1976).
(13) J. W. Sigler, A. S. Ridolfo, and G. B. Bluhm, *ibid.*, **3**, 49 (1976).
(14) J. F. Fries and M. C. Britton, *ibid.*, **3**, 61 (1976).
(15) M. Franke and G. Manz, *Curr. Ther. Res.*, **21**, 43 (1977).
(16) J. D. Davis, R. A. Turner, R. L. Collins, I. R. Ruchte, and J. S. Kaufman, *Clin. Pharmacol. Ther.*, **21**, 52 (1977).
(17) "Scientific Tables," 7th ed., Documenta Geigy, p. 712.
(18) J. F. Nash, R. J. Bopp, and A. Rubin, *J. Pharm. Sci.*, **60**, 1062 (1971).
(19) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 670.
(20) A. Rubin, B. E. Rodda, P. Warrick, A. S. Ridolfo, and C. M. Gruber, *J. Pharm. Sci.*, **61**, 739 (1972).
(21) *Ibid.*, **60**, 1797 (1971).
(22) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, p. 57.
(23) R. Ruckel, E. Forchielli, K. Sevelius, M. Chaplin, and E. Segra, *Clin. Pharmacol. Ther.*, **15**, 261 (1974).

ACKNOWLEDGMENTS

The authors thank Dr. F. C. Blubaugh for preparation of the capsules and Mr. P. R. Page for analysis of the plasma and urine samples.

Cholesterol Solubility in Organic Solvents

G. L. FLYNN *x, Y. SHAH *, S. PRAKONGPAN *, K. H. KWAN *, W. I. HIGUCHI *, and A. F. HOFMANN ‡

Received August 9, 1977, from the **College of Pharmacy, University of Michigan, Ann Arbor, MI 48109,* and the ‡*School of Medicine, University of California, San Diego, CA 92103.* Accepted for publication March 1, 1979.

Abstract □ The 37° cholesterol solubilities in over 50 solvents, including the homologous *n*-alkanols through dodecanol and homologous ethyl carboxylates through the undecanoate, and the 37° β-sitosterol solubilities in the *n*-alkanols through decanol are reported. Additionally, solubility data for cholesterol at 7, 17, and 27° in the alcohol series were obtained. These measurements allowed the calculation of heats of solution for cholesterol in the alkanols, which range from 7.5 kcal for methanol to 4.3 kcal for decanol and which tend to decrease, although irregularly, with increasing alkanol chain length. A solubility maximum in all of these series for both solutes was observed between a chain length of six and seven. A surprisingly irregular, odd–even alternating solubility pattern was noted for cholesterol in the alkanols at all four temperatures. Experimental evidence indicated that this pattern was due to solvent-induced crystalline changes, presumably solvate formation, in each alkanol solvent through C_{10}. Overall, the solubility studies screened solvents for their utility in dissolving cholesterol and, thus, cholesterol gallstones. To these ends, some limited dissolution experiments were performed, which indicated that the solution rate is directly related to the measured solubility in organic solvents. The dissolution behavior is thus different from micellar bile salt solutions, in which a significant interfacial barrier controls kinetics.

Keyphrases □ Cholesterol—solubility in organic solvents, *n*-alkanols, ethyl carboxylates, structure–activity relationships □ β-Sitosterol—solubility in organic solvents, *n*-alkanols, structure–activity relationships □ Gallstones—cholesterol, solubility in organic solvents, treatment of stones retained in common bile duct □ Organic solvents—cholesterol dissolution, treatment of gallstones retained in common bile duct, structure–activity relationships

Cholesterol is an important biological membrane constituent and is the principal component of many gallstones. It also has been indicted as a causative agent in arteriosclerosis. The physicochemical properties of cholesterol are important to its necessary membrane functions, to its role as an initiator and component of gallstones, and to its presumed involvement in vascular diseases. Indeed, the understanding of cholesterol's natural and pathophysiological roles is dependent on a thorough knowledge of cholesterol's biochemistry and physical chemistry. Particularly important is cholesterol solubilization in the major aqueous biological fluids, blood and bile (1–3). Elucidation of cholesterol's biological solubility and deposition would be aided by an understanding of its solubility behavior *in vitro*.

BACKGROUND

A promising direct application of cholesterol solubility data is the dissolution of small cholesterol stones in the common bile duct of patients

0022-3549/79/0900-1090$01.00/0

Table I—Cholesterol Solubility in the Alkanols at Several Temperatures

Alcohol	Temperature	Solubility, mg/ml[a] Radiolabel Assay	Solubility, mg/ml[a] Spectrophotometric Assay
Methanol	7°	2.30 (2.35, 2.25)	1.96
	17°	3.37 (3.44, 3.30)	3.65
	27°	6.09 (6.03, 6.15)	5.21 (5.38, 5.03)
	37°	7.89 (3, 0.26)	6.19 (4, 0.75)
Ethanol (95%)	7°	11.5 (11.6, 11.4)	11.0
	17°	17.0 (17.2, 16.7)	17.0
	27°	23.7 (23.5, 23.8)	23.4 (22.6, 24.2)
	37°	32.5 (3, 0.58)	31.1 (4, 6.29)
n-Propanol	7°	28.6 (28.5, 28.7)	28.0
	17°	41.0 (41.1, 40.9)	47.2
	27°	58.6 (59.4, 57.8)	63.6 (69.7, 57.4)
	37°	92.7	94.4 (4, 1.63)
n-Butanol	7°	31.6 (31.5, 31.7)	28.5
	17°	44.6 (44.5, 44.7)	46.1
	27°	64.2 (65.2, 63.1)	70.8 (65.6, 75.9)
	37°	95.1 (92.3, 97.8)	97.8 (4, 5.74)
n-Pentanol	7°	54.7 (53.3, 56.0)	47.6
	17°	79.5 (80.0, 78.9)	87.3
	27°	107.2 (108.4, 106.0)	121.5 (112.5, 130.4)
	37°	157.1 (158.3, 155.9)	150.0 (4, 1.40)
	37° (water saturated)	—	124
n-Hexanol	7°	69.3	62.3
	17°	91.7 (90.7, 92.6)	100.5
	27°	122.3 (3, 2.75)	143.3 (149.1, 137.4)
	37°	165.7 (165.9, 165.5)	178.2 (4, 9.63)
	37° (water saturated)	—	150.8
n-Heptanol	7°	91.1 (90.7, 91.5)	89.3
	17°	126.9 (129.0, 124.8)	139.0
	27°	157.9 (161.9, 153.9)	197.7 (191.0, 204.4)
	37°	210.9 (208.7, 213.1)	200.4 (4, 13.36)
	37° (water saturated)	—	176.9
n-Octanol	7°	74.2 (73.5, 74.8)	68.4
	17°	101.4 (103.9, 98.8)	103.9
	27°	128.8 (128.1, 129.4)	154.9 (154.0, 155.8)
	37°	172.4 (174.2, 170.5)	180.0 (4, 6.16)
n-Nonanol	7°	72.6 (73.6, 71.5)	—
	17°	96.7 (98.2, 95.2)	94.5
	27°	119.0	150.0 (144.7, 155.8)
	37°	164.0 (166.5, 161.5)	177.6 (4, 8.71)
	37° (water saturated)	—	182.9
n-Decanol	7°	98.3 (98.6, 97.9)	—
	17°	143.7 (140.5, 146.8)	142.7
	27°	168.7 (165.8, 171.5)	147.7 (152.7, 142.7)
	37°	212.2 (216.3, 208.0)	215.6 (4, 20.89)
n-Undecanol	37°	—	233.7 (237.1, 230.3)
n-Dodecanol	37°	—	110.5
Isopropanol	37°	48.9 (39.6, 58.2)	—
Isobutanol	37°	96.3 (98.0, 94.5)	—
sec-Butanol	37°	123.4 (131.8, 115.0)	—
tert-Butanol	37°	19.9 (20.1, 19.6)	—

[a] The averaged result is reported where there were more than two determinations, followed by parentheses which indicate the number of determinations and the standard deviation. For two determinations, the average is followed by parentheses containing the individual values. A single determination is without parentheses.

who have undergone cholecystectomy. This occurs in up to 5% of patients after gallbladder surgery (4–6).

The common duct is often explored during cholecystectomy, and a T-tube is inserted into the common duct to keep it "decompressed" during healing. If stones are discovered before the T-tube is removed, various approaches are possible. Traditionally, the patient simply has been observed since such "retained" stones may pass or dissolve spontaneously and duct reexploration is dangerous. There also have been repeated reports of the direct instillation into the T-tube of organic solvents, such as ether or chloroform (7), which are excellent solvents for gallstones *in vitro*. This approach has been abandoned as too hazardous by most physicians.

In 1972, Way *et al.* (6) directly instilled a micellar sodium cholate solution into the common duct *via* the T-tube for stone dissolution, and this procedure has been used with moderate success in uncontrolled studies (8, 9). Some investigators reported success in uncontrolled studies with micellar sodium chenodeoxycholate solutions, and others have proposed the instillation of presumably less toxic solvents, such as polyoxyethylene hydrogenated castor oil (10), or *N*-alkylacetamides (11). Recently, direct stone aspiration by a Dormia basket catheter has been the treatment of choice. Nonetheless, direct physical extraction of retained common duct stones is not always possible, and a need persists for a solution that can be directly infused into the common duct and that will safely induce rapid gallstone dissolution.

The present study is concerned with the solubility of cholesterol "monohydrate," a model gallstone material, in several homologous solvent series and in other selected solvents, some of which are promising from the standpoint of physiological suitability for dissolving cholesterol stones. The homologous series were chosen to provide regular alteration of the physicochemical properties of the solvent. Initially, it was felt that, if the optimum solvent polarity for cholesterol could be put in terms of a maximum in solubility in a homologous series of solvents, a rational basis for solvent selection would materialize. In other words, it was anticipated that solvents of the same solubility parameter or polarity as the best homolog also would be efficient solubilizers.

This approach was viewed as more systematic than most previous investigations, which reported on cholesterol solubility in fats, oils, arbitrarily selected organic solvents, and modified bile solutions (1–3, 12). During the present investigations on the cholesterol solubility in the *n*-alkanols, there appeared a physicochemical curiosity, an odd–even alternation in solubility at chain lengths up to seven carbons. Resolution of this phenomenon is a principal feature of this report.

EXPERIMENTAL

Preparation of Cholesterol Monohydrate—Crude anhydrous cholesterol (25 g) was dissolved in 2 liters of 95% ethanol warmed to 60°

Table II—Cholesterol Solubility at 37° in Various Organic Solvents

Solvent	Solubility, mg/ml[a] Radiolabel Assay	Spectrophotometric Assay
Ethylene glycol	0.30 (0.35, 0.24)	—
Propylene glycol	1.79 (1.51, 2.06)	2.07 (1.26, 2.88)
1,3-Butylene glycol	1.57 (1.56, 1.58)	3.32 (3.36, 3.27)
1,4-Butylene glycol	—	2.15
2,3-Butylene glycol	6.33 (7.52, 5.13)	5.27
Formamide	—	1.41
N-Methylformamide	5.33 (5.72, 4.93)	—
N,N-Dimethylformamide	—	4.92
Propylene carbonate	1.75 (1.75, 1.75)	—
Polyethylene glycol 400	3.85 (3.98, 3.72)	3.53 (3, 0.37)
Diethylene glycol monoethyl ether	53.3 (3, 0.36)	50.0
Diethylene glycol monoethyl ether acetate	29.5	—
Diethylene glycol monobutyl ether	106.5	—
2-Methoxyethanol	27.1 (26.1, 28.1)	—
2-Ethoxyethanol	87.0 (89.3, 84.6)	—
2-Methoxyethyl acetate	28.5 (28.5, 28.5)	—
2-Ethoxyethyl acetate	43.2	—
2-(2-Ethoxyethoxy) ethyl acetate	30.3 (3, 0.85)	—
2-(2-*n*-Butoxyethoxy) ethanol	106.9 (106.5, 107.2)	—
Dipropylene glycol	25.6	29.7
Ethylene glycol diacetate	7.01 (3, 0.44)	6.90
Glyceryl triacetate (triacetin)	7.17	—
Methyl benzoate	107.9 (3, 1.82)	—
Glyceryl-1-monooctanoate (monooctanoin)	116.8	—
Glyceryl trioctanoate (tricapryline)	52.0 (3, 0.79)	—
Isopropyl myristate	67.4 (67.7, 67.1)	—
Oleic acid	146.2 (3, 8.45)	—

[a] The averaged result is reported where there were more than two determinations, followed by parentheses which indicate the number of determinations and the standard deviation. For two determinations, the average is followed by parentheses containing the individual values. A single determination is without parentheses.

and filtered through a paper filter[1] while warm. This mixture was allowed to return to room temperature and to crystallize by standing overnight. The formed crystals were collected in a sintered-glass funnel and dried 15–18 hr under vacuum. The material was crystallized a second time using the same procedure. The twice-crystallized cholesterol was stored in the dark in a desiccator containing water (100% humidity).

Radiolabeled cholesterol was prepared similarly. After the first crystallization, 25 g of cholesterol was dissolved in 2 liters of 95% ethanol at 65°, 10 μCi of ^{14}C-cholesterol in benzene (<1 ml) was added, and the recrystallization was carried out as with the cold material. The activity of the labeled cholesterol was 803 cpm/mg.

The other materials were organic solvents obtained commercially[2] in the purest form available and were used as received.

Sample Preparation—Excess cholesterol monohydrate was placed in screw-capped test tubes containing 3–4 ml of solvent. The tubes were sealed and placed in a thermostated bath (7, 17, 27, or 37°) equipped with a shaker and equilibrated for ≥3 days. In preliminary studies, this time was more than adequate to obtain equilibrium. Samples were taken using glass wool-tipped pipets preconditioned to the experimental temperature. The samples were appropriately diluted for the spectrophotometric procedure using 95% ethanol. In the radiolabel assay, the samples were added directly to the scintillation cocktail without further dilution.

Cholesterol solubility in water-saturated, water-immiscible *n*-alkanols was also determined (C_5 and above). In this case, excess cholesterol was added to a two-phase system containing several milliliters of an aqueous phase overlayered with several milliliters of the immiscible, water-saturated alkanol phase. The two-phase systems were equilibrated for ≥3 days with gentle agitation and allowed to reform into two clear, distinct layers. Samples were drawn carefully from the upper organic phase using glass wool-tipped pipets. These samples were assayed using the procedures employed with the pure solvents.

[1] Whatman.

[2] Aldrich; Baker; Eastman; Fisher; Matheson, Coleman, and Bell; and U.S. Industrial Chemicals.

Table III—Cholesterol Solubility in the Homologous Ethyl Carboxylates at 37°

Ethyl Carboxylate Solvent	Solubility[a], mg/ml (37°, Radiolabel Assay)
Ethyl acetate	76.4
Ethyl propionate	101.4 (101.3, 101.5)
Ethyl butyrate	103.0 (102.6, 103.3)
Ethyl pentanoate (valerate)	112.3
Isoamyl acetate	109.5 (109.9, 109.1)
Ethyl hexanoate	101.9 (3, 2.55)
Ethyl octanoate	99.2 (98.6, 99.7)
Ethyl nonanoate (pelargonate)	95.6 (3, 1.46)
Ethyl deconoate	88.5 (87.4, 89.5)
Ethyl undecanoate	102.9 (3, 3.72)
Ethyl dodecanoate (laurate)	85.2
Ethyl tetradecanoate (myristate)	78.5
Ethyl hexadecanoate (palmitate)	76.7

[a] The averaged result is reported where there were more than two determinations, followed by parentheses which indicate the number of determinations and the standard deviation. For two determinations, the average is followed by parentheses containing the individual values. A single determination is without parentheses.

Generally, duplicate samples were taken from each solute–solvent equilibrium mixture at separate times and were assayed independently. The initial studies were done using the spectrophotometric procedure. However, as can be seen from Tables I and II, this procedure was accompanied by substantial variability, so an alternative procedure involving radiolabeled cholesterol was adopted. This procedure proved to be far more precise, although the averaged results between procedures were in reasonable agreement for the alkanols and some other solvents. With few exceptions, replicate assays with the radiolabeled cholesterol agreed within 5%. Specific statistical data on the methods are given in Tables I–III. Percent coefficients of variation calculated from these data were typically between 1 and 5% and were smaller for the least viscous solvents, such as the low molecular weight alcohols.

Given the unusual solubility patterns observed for cholesterol in the *n*-alkanols, the solubilities of a structurally closely related steroid, β-sitosterol, in these normal alcohol solvents were determined also. This experiment was done to see if the peculiar solubility behavior was unique to cholesterol or was a more general phenomenon exhibited by this steroid class. The procedure was identical to that employed in the cholesterol analysis. Radiolabeled β-sitosterol was recrystallized from ethanol for the experiment.

Radiolabel Assay—Scintillation cocktail was made as follows: naphthalene, 60 g; 2,5-diphenyloxazole, 4 g; 1,4-bis[2-(5-phenyloxazolyl)]benzene, 0.2 g; absolute methanol, 100 ml; ethylene glycol, 20 ml; and dioxane, *qsad*, 1000 ml.

Aliquots of 0.1 ml were taken from the saturated solutions and diluted in 10 ml of the cocktail. In all cases, clear solutions were obtained. The vials were placed in a scintillation counter, and the disintegrations (counts) per minute were obtained. These values were compared against standard solutions prepared by taking 0.5 ml of a cholesterol solution in acetone containing 114.9 mg of labeled compound/50 ml, evaporating it to dryness, and dissolving the residue in 10 ml of the cocktail. It was unnecessary to add 0.1 ml of the particular solvent to simulate the sample composition. The solubility in milligrams per milliliter was calculated from the ratio of the sample counts to the standard counts, times the appropriate dilution factor.

Spectrophotometric Procedure—Cholesterol solubility in the *n*-alkanols and some other polar solvents was determined by a literature spectrophotometric procedure (13) (cold cholesterol) as well as by scintillation counting, which was used for most solvents. The spectrophotometric procedure was used for the initial work, but the data are presented here only as a cross-check on the scintillation method. The reader is referred to the literature for the details of this complex assay. Where both analytical procedures were employed, the results were in close agreement.

Differential Thermal Analysis—The equilibrium crystals in the *n*-alkanols were collected on a sintered-glass funnel and rinsed quickly with about 50 ml of cold hexane to remove residual alcohol. The crystals were transferred to a filter paper and allowed to dry overnight at room temperature. Several milligrams of cholesterol obtained from each alcohol was placed in glass capillaries, and differential thermal scans[3] were made. All tracings were repeated, and no significant differences appeared in the thermograms from run to run with a given compound.

[3] Du Pont 990 thermal analyzer.

Table IV—Cholesterol Solubilities in Binary Mixtures of *n*-Alkanols at 37° Adjusted to Fractional Chain Lengths

Fractional Chain Length	Alkanol Pair	Solubility, mg/ml: Radiolabel Assay	Solubility, mg/ml: Spectrophotometric Assay
2.5	Ethanol–propanol	—	67.0
3.5	Propanol–butanol	—	100.5
4.5	Butanol–pentanol	—	127.1
5.5	Pentanol–hexanol	—	189.9
6.1	Hexanol–heptanol	183.4	—
6.2	Hexanol–heptanol	192.9	—
6.3	Hexanol–heptanol	202.0	—
6.4	Hexanol–heptanol	212.0	—
6.5	Hexanol–heptanol	215.0	114.6 (?)[a]
6.6	Hexanol–heptanol	216.2	—
6.7	Hexanol–heptanol	219.9	—
6.8	Hexanol–heptanol	215.9	—
6.9	Hexanol–heptanol	214.2	—
7.5	Heptanol–octanol	—	198.3
8.5	Octanol–nonanol	—	206.5

[a] This data point was discarded as an anomaly.

RESULTS

Cholesterol solubilities in the *n*-alkanols from methanol to undecanol are given in Table I. These values were determined at 7, 17, 27, and 37° for most of the pure alkanols and at 37° in several water-saturated long chain alkanols. Both the spectrophotometric procedure and radiolabeled assay were used and gave results generally in accord.

The data for the alkanol solvents through chain length 10 at the four temperatures are plotted in Fig. 1. This profile is characterized by an odd–even alternation in solubility up to an alkanol solvent chain length of seven, followed by an erratic solubility pattern through the remainder of the series. The "staircase effect" is somewhat more pronounced at the higher temperatures.

The solubilities of cholesterol at 37° in a second homologous series, the ethyl carboxylates, were also determined (Table III). Data are not available for the C_7 compound, but otherwise the series is uninterrupted from C_2 to C_{11}; ethyl heptanoate was not available from the major

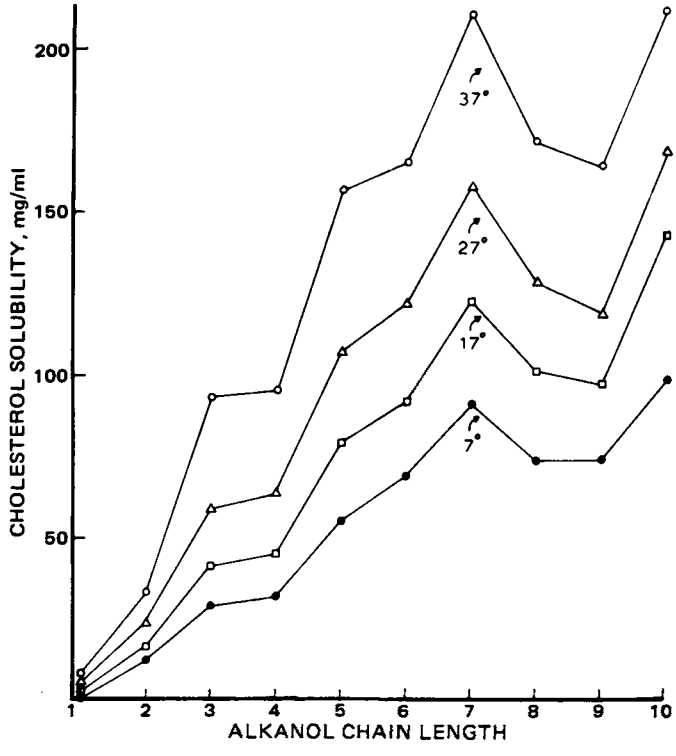

Figure 1—*Solubility profiles of cholesterol in the* n*-alkanols at 7, 17, 27, and 37°. The unusual feature of these plots is the odd–even alternation in solubility behavior from C_1 to C_7. It is apparent from thermal analysis data that the equilibrium crystals yielding these solubilities are not identical. The plotted data are those obtained with the radioassay.*

Table V—β-Sitosterol Solubilities in the *n*-Alkanols at 37°

Alcohol Solvent	Solubility, mg/ml[a] (Radiolabel Assay)
Methanol	5.39 (5.34, 5.44)
Ethanol	36.3 (37.0, 35.6)
n-Propanol	204.8 (209.0, 200.5)
n-Butanol	279.8 (294.4, 265.2)
n-Pentanol	318.6 (312.2, 324.9)
n-Hexanol	333.1 (326.2, 339.9)
n-Heptanol	312.1 (312.2, 312.0)
n-Octanol	315.1 (314.8, 315.3)
n-Nonanol	314.9 (318.2, 311.6)
n-Decanol	319.3 (314.6, 324.0)

[a] The parentheses indicate the values of the independent determinations.

chemical suppliers. The solubility of the isoamyl acetate is listed in the table under that of the comparable compound, ethyl valerate. These two compounds have the same molecular weight and empirical formula, $C_7H_{14}O_2$, and their solubilizing capacity for cholesterol is nearly identical.

The data found in Table III are also displayed in Fig. 2. Unlike the alkanols, the solubility–chain length profile is a smooth curve. The maximum in the plot is observed at a carboxyl chain length of five or total carbons, counting the ethyl ester group, of seven. The solubility varies only about 50% from the minimum to the maximum in the series.

The 37° solubilities of cholesterol in diverse additional solvents are given in Table II. These values are crudely grouped according to the type of solvent structure and are listed in rough progression from polar to nonpolar solvent character.

The cholesterol solubilities in binary *n*-alkanol mixtures combined so as to give a "fractional chain length" were also determined (Table IV) by mixing appropriate amounts of adjacent homologs to predetermined fractional molar compositions, usually half and half. A mixture containing equal molar amounts of ethanol and propanol, for instance, was considered to have an effective chain length of 2.5. Because of an initial analytical error, which indicated that the solubility of fractional chain length 6.5 was approximately one-half of the value expected based on the solubilities of pure hexanol or heptanol, the cholesterol solubility in binary solvent mixtures of these two alcohols was studied in detail (Fig. 3). There is a maximum in solubility at about fractional chain length 6.7, but the solubility in this mixture is only slightly greater than that observed in pure heptanol.

Lastly, the solubilities of β-sitosterol were also determined in the *n*-alkanols (Table V and Fig. 4). The solubility patterns observed are totally

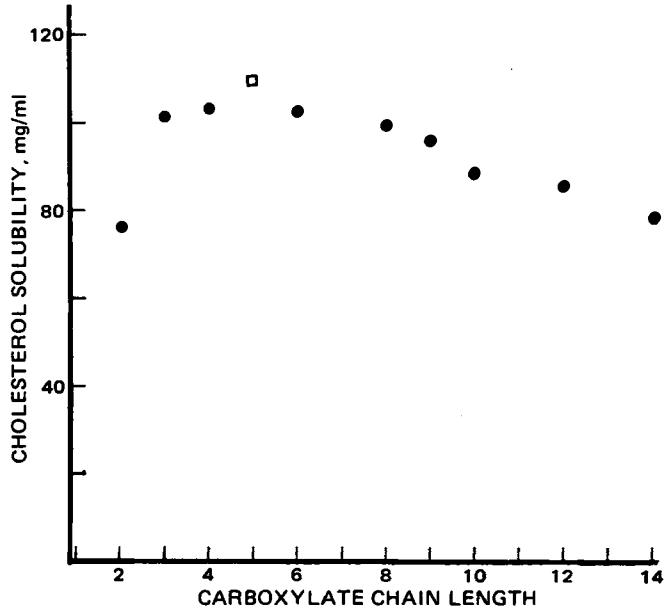

Figure 2—*Solubility at 37° of cholesterol monohydrate in the homologous ethyl carboxylates. Unlike the* n*-alkanols, the curve is without serious irregularities, suggesting that there are no solvent-induced crystalline changes in these solvents. Key: □, isoamyl acetate.*

Table VI—Heats of Solution of Cholesterol in the *n*-Alkanols through Decanol

Alcohol Solvent	Least-Squares Slope Arrhenius Plot[a]	Correlation Coefficient	Heat of Solution, ΔH_s, cal/mole
Methanol	-1.648×10^3	−0.9926	7540
Ethanol	-1.301×10^3	−0.9997	5950
n-Propanol	-1.464×10^3	−0.9961	6700
n-Butanol	-1.383×10^3	−0.9983	6330
n-Pentanol	-1.304×10^3	−0.9986	5970
n-Hexanol	-1.095×10^3	−0.9989	5010
n-Heptanol	-1.042×10^3	−0.9993	4770
n-Octanol	-1.045×10^3	−0.9991	4780
n-Nonanol	-0.9995×10^3	−0.9962	4570
n-Decanol	-0.9327×10^3	−0.9878	4270

[a] Slope = $(-\Delta H_s/2.303R)$.

unlike those seen for cholesterol and indicate that cholesterol is unique in the way it interacts with the homologous alcohols.

DISCUSSION

General Solubility Patterns—The 37° solubility data for cholesterol in the ethyl carboxylates (Table III and Fig. 2) and in the diverse solvents listed in Table II suggest some general solubility patterns. Not too surprisingly, cholesterol is relatively insoluble in the very polar solvents such as the glycols and formamides. The solubility in ethyl acetate of 76.4 mg/ml is the lowest observed in the ethyl carboxylate series, although it is not experimentally different from the solubility in ethyl myristate (78.5 mg/ml) and ethyl palmitate (76.7 mg/ml).

The solubility in the latter two solvents suggests that they are effectively more nonpolar than cholesterol and interact less strongly with this compound than the intermediate chain length esters. Since the intermolecular forces involved here would be primarily of the London dispersion and other van der Waals types, it can be qualitatively stated that the solubility parameter of the shortest and longest chain esters differs from that of cholesterol and that there is a significant activity coefficient correction based on the solubility parameter differential, in the direction of reducing the solubility. It is assumed here that there are no significant

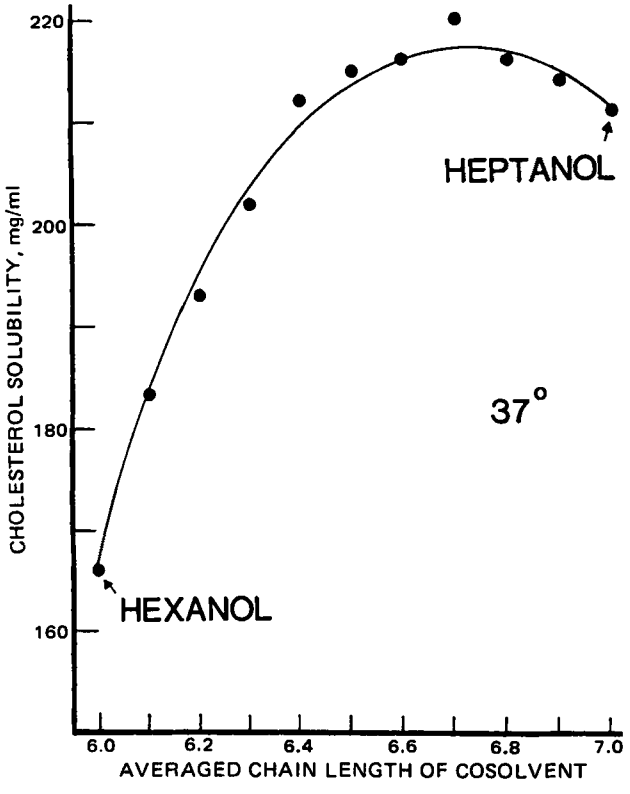

Figure 3—*Cholesterol solubilities in mixtures of hexanol and heptanol against the average chain length of the solvent pair. The extreme points are the solubilities in pure hexanol and heptanol.*

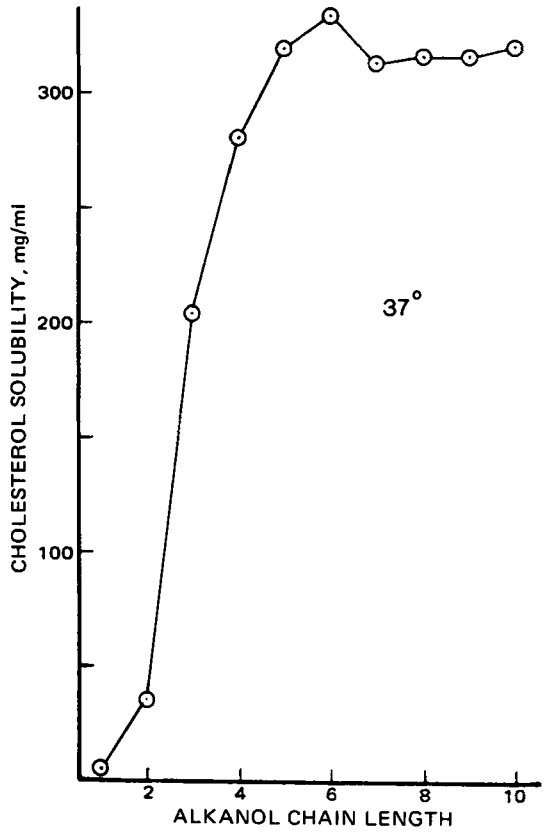

Figure 4—*Solubility of β-sitosterol in the n-alkanols. The behavior here is far more regular than that seen for cholesterol in these same solvents.*

crystalline changes in these solvents. Certainly, the smooth solubility profile (Fig. 2) strongly suggests this condition.

These general observations are fully consistent with those of previous investigators (2) who studied cholesterol solubility in various fats and oils and in a series of pure triglycerides. In general, they found the solubility in oils not to vary appreciably, as observed with the ethyl carboxylates, but they also found the solubility to be greatest in fats and oils containing relatively short chain (C_6–C_{12}) fatty acids. In the pure triglycerides, they observed a solubility maximum of 54.7 mg/ml for glyceryl tridecanoate (tricaprin). Solubilities were considerably less in short chain triglycerides and somewhat less in triolein and trilinolein, 37.7 and 34.1 mg/ml, respectively. The reported (12) very low solubility of cholesterol in mineral oil, an apolar solvent, of 13.9 mg/ml at 37° also is consistent with the observation that a moderate polarity favors solubility. The consistency of cholesterol solubility in ethyl valerate (112.3 mg/ml), isoamyl acetate (109.5 mg/ml), and methyl benzoate (107.9 mg/ml), which are all C_7 esters, further indicates a general cohesive energy effect because these esters would have similar solubility parameters.

Another factor affecting solubility, in addition to the general cohesive energy effect, is evident from consideration of several pairs of compounds. The cholesterol solubility in 2-(2-butoxyethoxy)ethanol (106.9 mg/ml) was more than threefold greater than that found for 2-(2-ethoxyethoxy) ethyl acetate (30.3 mg/ml). The same was true for diethylene glycol monobutyl ether (106.5 mg/ml). These two pairs have similar empirical formulas. Cholesterol solubility in glyceryl-1-monooctanoate was over twice that for glyceryl trioctanoate, which may be a solubility parameter effect. Solubility in diethylene glycol monoethyl ether was considerably greater than in diethylene glycol acetate, which is in the opposite direction from the general trend. Solubility in 2-ethoxyethanol was slightly greater than in ethyl acetate, which also runs contrary to the general polarity behavior.

In each pair, the better solubilizing solvent had a free hydroxyl group, two in the case of the glyceride, but the hydroxyls were esterified in the poorer solvent pair member. The presence of a free hydroxyl obviously favors solution. Whether the effect is due to enhanced intermolecular interaction in the solution phase or whether it is due to a change in the cholesterol crystal form occurring in the approach to equilibrium, or both, is undetermined. Energetic hydrogen bonding with the cholesterol hy-

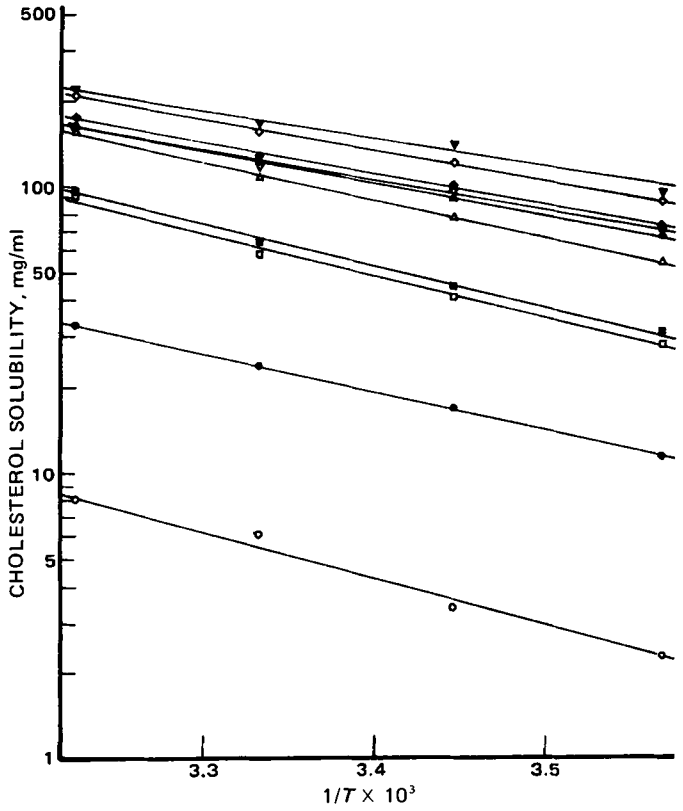

Figure 5—*Arrhenius-type plot of the solubilities of cholesterol in the n-alkanols. Key: ○, methanol; ●, ethanol; □, propanol; ■, butanol; △, pentanol; ▲, hexanol; ◇, heptanol; ◆, octanol; ▽, nonanol; and ▼, decanol.*

droxyl is certainly promoted by having free hydroxyl functions in the solvent structure. However, as will be demonstrated, some alcohols also change the nature of the crystalline phase.

In most solvents, even the poorer ones, cholesterol solubility exceeds that found in micellar bile acid solutions. Typically, the solubility in the latter systems is ~0.5–1.5 mg/ml (9). Given that the dissolution rate of an organic solid is generally directly proportional to its solubility in the solvent, this observation superficially suggests that organic solvents should be more efficient (in terms of rates) in dissolving gallstones. The superficiality of this statement resides in the fact that gallstone dissolution is controlled by an interfacial barrier, at least with respect to bile acid solubilization, and is not diffusion controlled and also in the fact that the dissolution process hydrodynamics have not been accounted for. If the solvent is viscous, the solution rates will be slowed because a thicker effective boundary layer will form around the stone and because the diffusivity in this layer may be significantly reduced. Nevertheless, cholesterol solubility in many of these solvents is sufficiently high to suggest that residual stones may be dissolved and removed relatively quickly by solvent dissolution.

Cholesterol Solubility in *n*-Alkanols—The introduction stated that the *n*-alkanols were chosen as model solvents to pinpoint an optimum solvent polarity for cholesterol, at least qualitatively, to put solvent selection for gallstone dissolution on a rational basis. These solvents show regular incremental increases in their physicochemical properties, such as boiling points, heats of vaporization, and water solubilities, all related to the alkyl chain length. The use of regularly altering physicochemical properties has proved fruitful in other studies involving organizing and interpreting solubilities of homologous solutes in a given solvent (14) and solubilities of a given solute in binary solvent mixtures (15). As can be seen in Fig. 1, cholesterol solubilities in the alkanols do not yield a smooth solubility trend as a function of solvent chain length. Rather, the solubility profile is irregular and exhibits odd–even alternations up to heptanol.

Heats of solution for each compound also were obtained from plots of the solubility logarithms *versus* reciprocal absolute temperature (Fig. 5). These values systematically decrease with increasing solvent alkyl chain length (Table VI), albeit irregularly. The value for ethanol, which

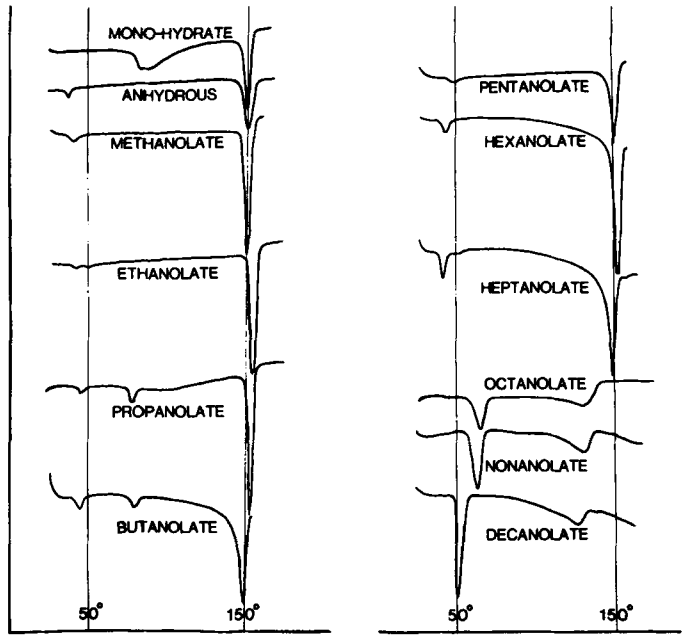

Figure 6—*Differential thermal analysis tracings of the equilibrium cholesterol crystals from the various n-alkanols as well as of the monohydrate and anhydrous forms. All curves were reproducible for a given solvent and decidedly different between solvents. The 50 and 150° reference points are given. The tracings are identified as if for true solvates but are for crystalline forms, which are not as yet fully characterized.*

appears to fall out of the overall trend, was obtained in 95% ethanol and not in the pure solvent.

It was reasoned that if the general solvent properties were sensitive to whether the alcohol was odd or even in carbon number, which would be over and above more general effects of increasing alkyl chain length, then the solubility behavior of closely related steroids should parallel that shown for cholesterol. Therefore, the solubility of β-sitosterol in the alkanols was examined. The data (Fig. 4) form a smooth profile, which plateaus at about 320 mg/ml between C_5 and C_{10}. While the very large solubilities at the longer chain lengths are unusual for this type of compound, clearly the behavior is dissimilar to that of cholesterol, ruling out a nonspecific solvent effect. Also, since the odd–even effect was seen at four temperatures, it was not the result of a solid phase transition known to occur at 37° (16).

The unusual solubility behavior was resolved by collecting the equilibrated cholesterol crystals in each alkanol and examining the solid by qualitative differential thermal analysis. Figure 6 shows thermogram tracings for cholesterol crystals obtained from saturated solutions of each alkanol along with a tracing of the starting material, cholesterol monohydrate. The fusion properties of the solvent-equilibrated crystalline phases are quite different. These data indicate that cholesterol "solvates" [4] of each alkanol are formed and, taken together with the solubilities, further suggest that the crystalline properties of the formed solvates exhibit alternations in the fusion energetics, which are effectively odd–even in character up to a chain length of seven. This phenomenon is typical of the fusion energetics of homologs (17) but apparently has never been demonstrated for solvates.

The thermogram tracing for the starting material, cholesterol monohydrate, was rerun multiple times and was never exactly the same. Its general features were a shallow endotherm beginning at about 80° with a double minimum. From run to run, the minimums shifted slightly on the temperature axis and alternated in relative magnitude. Furthermore, the major melting endotherm was double tipped in most cases with minimums at ~151 and 152.5°. There is a strong suggestion that this is not a pure crystal but a mixture of forms, probably, considering the re-

[4] The term "solvate" is used here with some liberty. The thermograms show the crystalline phases to be different, but the exact nature of the differences is unknown. However, the solubility patterns strongly suggest solvent participation in the crystalline form. Other data in the literature concerning cholesterol solid states support this idea (16).

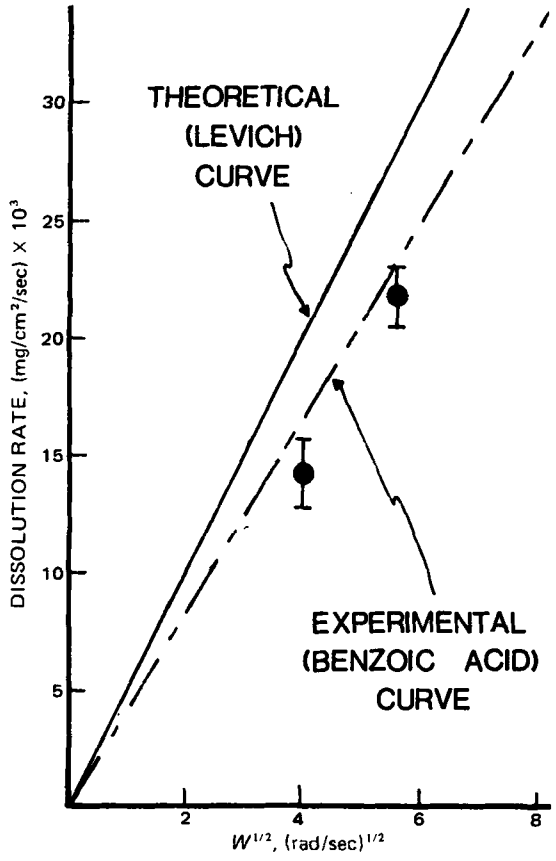

Figure 7—*Theoretical behavior for dissolution from a rotating flat surface according to Levich (—) and the expected behavior for the dissolution cell based on benzoic acid dissolution (- - -). The dissolution of cholesterol at 150 and 300 rpm falls very near the exact expectations for boundary layer control based on the benzoic acid "calibration."*

crystallization procedure and storage conditions, an ethanolate and a hydrate.

On cooling and remelting, the curve took a different form with a slight endotherm at 37° and a major melting endotherm at 151°. This curve has been associated with anhydrous cholesterol (16). The 37° endotherm is a solid phase transition known to occur with the anhydrous form (16). In methanol-equilibrated crystals, there is also an endotherm at ~40° (minimum) and the retracing on cooling is indistinguishable from that of the anhydrous form. In ethanol, there is a broad and very shallow endotherm beginning at ~40° and extending beyond 50°. In propanol, there is an initial shallow endotherm at 45° and a second shallow endotherm at ~78°. All three of these alkanols exhibit a major melting endotherm at 151°. All yield the same thermogram tracing after cooling and remelting, characteristic of the anhydrous form.

The thermograms for butanol-, pentanol-, hexanol-, and heptanol-equilibrated crystals exhibit differences in the magnitude and placement of the minor endotherm but all also melt essentially at 150°. In these solvates, there is no return to the anhydrous form based on thermogram retracings. For octanol, nonanol, and decanol, the entire thermogram shape is changed; in each case, the major endotherm is found below 70° or at 65, 63, and 50°, respectively. In each case, there also is a peculiarly shaped endotherm above 100°. The change in melting behavior seen at octanol and above corresponds with changes in solubility patterns seen in the solubility profile above C_7.

Just as in the case of the ethyl carboxylates (counting all carbons), there is a maximum in the alkanol solubility at C_7. The alcohols also provide a hydroxyl for intermolecular bonding with the cholesterol hydroxyl and, as seen in the previous analysis, this feature seems to be important in inducing solubilization. Thus, it appears that the larger cholesterol solubilities in the intermediate chain length alkanols can be attributed to the proper polarity, the presence of a free hydroxyl, and modifications in the crystal form in equilibrium with the saturated phase. From the data in Fig. 3, the ideal polarity appears to be slightly less than that of heptanol; a solubility maximum exists in binary mixtures of hexanol and heptanol. If the data are examined carefully, however, the fractional chain length solubilities are, without exception, larger than the average of the bracketing pure solvents, and this is probably attributable to mixed crystalline forms.

The study presented here, when considered in its entirety, suggests a general caution to investigators dealing with solubility analysis. Solubility data are often collected and analyzed with the assumption of solid phase stability. These experiences with cholesterol indicate that the solid phase for some compounds may be remarkably sensitive to the solvents used. Such effects were observed previously (18) for corticosteroids. Thus, before one can *quantitatively* analyze solubility data, the solid phase properties must be examined and shown to be invariant with the solvent system used. Otherwise, both crystalline energetics and solution phase energetics must be simultaneously accounted for. Other compounds with solvent-dependent crystalline forms probably will surface if the solid phases in equilibrium are examined routinely.

The ethyl carboxylates and glyceryl esters were studied because of their relative safety for use in the T-tube elution of cholesterol gallstones retained in the common bile duct. The ethyl carboxylates should be absorbed poorly due to their limited aqueous solubilities, and their hydrolysis products, ethanol and the respective long chain acids, also should be physiologically innocuous in moderate quantities. The glyceryl esters were presumed even more suited for this purpose from the toxicity standpoint. The solubilities in these solvents ranged from ~ 5% (w/v) to as much as 11% (w/v) for ethyl valerate and glyceryl monooctanoate. The latter was the most suitable for gallstone dissolution in view of its cholesterol-solubilizing capacity and its physiological compatibility.

The general approach followed with regard to solubility and its impact on cholesterol dissolution was based on the assumption that the solution rate is proportional to the solubility in the solvent, after due consideration to fluid hydrodynamics. Since this is not the case for cholesterol dissolution in bile acid solutions, in which a large rate-controlling interfacial barrier is operating (19), it seemed necessary to assess whether the presumed direct relationship between solubility and dissolution exists for organic solvents. Because of its apparent suitability for cholesterol stone dissolution in the common duct, a few dissolution experiments were performed with glyceryl monooctanoate. Compressed pellets of cholesterol monohydrate were used as the model cholesterol gallstone; the procedure, detailed in a previous paper (19), is based on dissolution from a rotating disk (20).

The dissolution apparatus was calibrated using benzoic acid, a substance known to follow diffusion layer-controlled dissolution kinetics. The kinematic viscosity of glyceryl monooctanoate and the diffusivity of cholesterol in glyceryl monooctanoate, both necessary for the analysis of the rotating disk dissolution experiment, were determined and estimated, respectively. The viscosity was 50.6 centipoises on a Cannon-Fenske-modified Ostwald viscometer. The density was 0.9954 g/cm^3, thus yielding a kinematic viscosity of 50.8 centistokes. The diffusivity was estimated to be 5.6×10^{-8} cm^2/sec using the Stokes–Einstein equation.

The data for the dissolution experiments are shown in Fig. 7, along with the experimental benzoic acid curve and the theoretical dissolution curve according to Levich (20). The data points for cholesterol dissolution in glyceryl monooctanoate at 150 and 300 rpm fall only slightly below the benzoic acid curve, indicating that cholesterol dissolution in this solvent is essentially diffusion layer controlled.

Since similar diffusion-controlled dissolution kinetics have been demonstrated for cholesterol monohydrate in a 63% ethanol–water mixture (21), it can be assumed that cholesterol dissolution in organic media is unlike that observed in micellar bile acid–lecithin media and generally will be diffusion layer controlled and predictably dependent on the solvent viscosity and the cholesterol solubility in the solvent. Thus, it seems reasonable to select solvents for T-tube perfusion of retained cholesterol stones on the basis of physiologic compatibility, capacity to solubilize cholesterol, and viscosity. Obviously, the greater the solubility and the smaller the kinematic viscosity, the faster is the dissolution rate.

REFERENCES

(1) J. A. Wilkens, J. A. deWit, and B. Bronte-Steward, *Can. J. Biochem. Physiol.*, **40**, 1091 (1962).

(2) D. Kritchevsky and S. H. Tepper, *Proc. Soc. Exp. Biol. Med.*, **116**, 104 (1964).

(3) R. D. Stauffer and F. Bischoff, *Clin. Chem.*, **12**, 206 (1966).

(4) C. Lansford, S. Mehta, and F. Kern, *Gut*, **15**, 48 (1974).

(5) F. Glenn, *Surg. Gynecol. Obstet.*, **134**, 249 (1972).
(6) L. Way, W. Admirand, and J. E. Dunphy, *Ann. Surg.*, **176**, 347 (1972).
(7) W. Walters and H. R. Wesson, *Surg. Gynecol. Obstet.*, **65**, 695 (1937).
(8) R. G. Danzinger, A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle, *N. Engl. J. Med.*, **286**, 1 (1972).
(9) A. M. Molokhia, W. I. Higuchi, and A. F. Hofmann, *J. Pharm. Sci.*, **64**, 2029 (1975).
(10) T. Hisatsugu, H. Igimi, and M. Nishimura, *Jpn. J. Surg.*, **2**, 62 (1972).
(11) H. Igimi, *Igaku Kenkyu*, **42**, 55 (1972).
(12) L. D. Wright and J. A. Presberg, *Proc. Soc. Exp. Biol. Med.*, **115**, 497 (1964).
(13) W. M. Sperry and M. Webb, *J. Biol. Chem.*, **123**, 97 (1951).
(14) G. Saracco and E. S. Marchetti, *Ann. Chim.*, **48**, 1357 (1958).
(15) S. H. Yalkowsky, G. L. Flynn, and G. L. Amidon, *J. Pharm. Sci.*, **61**, 983 (1972).
(16) H. L. Spier and K. G. VanSenden, *Steroids*, **6**, 871 (1965).
(17) S. H. Yalkowsky, G. L. Flynn, and T. G. Slunick, *J. Pharm. Sci.*, **61**, 852 (1972).
(18) J. A. Ostrenga and C. Steinmetz, *ibid.*, **59**, 414 (1970).
(19) S. Prakongpan, W. I. Higuchi, K. H. Kwan, and A. M. Molokhia, *ibid.*, **65**, 685 (1976).
(20) V. G. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962.
(21) S. Prakongpan, Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1974.

Sensitive Fluorescence Assay for *d,l*-Methadone

CHRISTINA H. CHI and BALWANT N. DIXIT ˣ

Received February 9, 1976, from the *Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication February 28, 1979.

Abstract □ *d,l*-Methadone forms a fluorophore when reacted with paraformaldehyde in concentrated sulfuric acid. Based on this reaction, a fluorescence assay suitable for quantitative *d,l*-methadone analysis from plasma and other tissues was developed. *d,l*-Methadone was extracted at pH 9.2 from the deproteinized filtrate of plasma or of aqueous tissue homogenate into an organic phase of 25% isobutanol in ethylene dichloride. After an aliquot of the organic phase was evaporated to dryness at 50–55° with an air jet, paraformaldehyde (0.1%, in concentrated sulfuric acid) was added, and fluorescence was read at 450 nm with excitation at 275 nm. By this method, *d,l*-methadone can be assayed in the presence of its metabolites, morphine, diacetylmorphine (heroin), codeine, and cocaine; however, amphetamine, meperidine, and quinine interfere.

Keyphrases □ Methadone—analysis, fluorescence assay, lung, liver, brain, serum, rats □ Narcotic analgesics—methadone, fluorescence assay, lung, liver, brain, serum, rats □ Fluorometry—analysis, methadone in lung, liver, brain, serum, rats

During the past decade, *d,l*-methadone has been extensively used in maintenance and rehabilitation programs for chronic diacetylmorphine (heroin) users (1). Most of the work on its distribution and metabolic disposition (2–4) has involved complex assays requiring labeled methadone. Other available methods are not sensitive enough to study *d,l*-methadone distribution following therapeutic doses (5–9). Recently, a highly sensitive GLC method (10) was reported.

When *d,l*-methadone is reacted with paraformaldehyde in concentrated sulfuric acid by heating at 100°, a fluorophore is formed (11). The purpose of this paper is to report a fluorescence assay based on this observation. This assay is applicable for methadone analysis from biological tissues in nanogram concentrations.

EXPERIMENTAL

Reagents—All reagents were prepared from reagent grade chemicals.

Zinc Sulfate Solution, 5%—Fifty grams of $ZnSO_4 \cdot 7H_2O$ was dissolved in glass-distilled water to a final volume of 1000 ml. The solution was filtered to remove any turbidity.

Barium Hydroxide Solution, 4.5%—Forty-five grams of $Ba(OH)_2 \cdot 8H_2O$ was dissolved in glass-distilled water to a final volume of 1000 ml. The solution was filtered to remove any turbidity and stored in tightly stoppered bottles.

Borate Buffer, pH 9.6—Fifty milliliters of 0.1 *M* boric acid in 0.1 *M* KCl was mixed with 36.85 ml of 0.1 *M* NaOH.

25% Isobutanol in Ethylene Dichloride—Isobutanol, 250 ml, was mixed with 750 ml of ethylene dichloride.

0.1% Paraformaldehyde in Sulfuric Acid—Paraformaldehyde, 25 mg, was dissolved in 25 ml of concentrated sulfuric acid. This reagent was prepared fresh just prior to use.

Procedure—Rats were pretreated with *d,l*-methadone and sacrificed by decapitation. Blood and other tissues such as lung, liver, and brain were removed. Plasma or serum was separated by centrifugation. Tissue samples were frozen over powdered dry ice and stored at −20° until assayed.

Just prior to assay, tissues were weighed and homogenized in distilled water (1:3). A 2-ml aliquot of the homogenate or 1 ml of serum (or plasma) diluted to 2 ml with distilled water was used for the assay. One milliliter of zinc sulfate solution was added to each sample, and the contents were thoroughly mixed on a vortex mixer before the addition of 1 ml of barium hydroxide solution. After thorough mixing, samples were centrifuged at 1000×*g* for 6 min[1].

Two-milliliter aliquots of the clear supernate were transferred into 15-ml glass-stoppered centrifuge tubes, and 0.4 ml of pH 9.6 borate buffer was added to give the final pH of 9.2. Six milliliters of 25% isobutanol in ethylene dichloride was added, and the tubes were shaken on a reciprocating shaker[2] for a minimum of 10 min to facilitate methadone extraction into the organic phase. The samples were centrifuged for 6 min at 1000×*g*, and the aqueous layer was removed by aspiration and discarded.

Three milliliters, or any other suitable aliquot of the organic phase, was then transferred to a set of test tubes, and the contents were evaporated to dryness under an air jet. The air was passed sequentially through a column of water and concentrated sulfuric acid. The test tubes were kept in a water bath maintained at 50–55°. Paraformaldehyde reagent, 0.1 ml, was added to each sample, and the tubes were transferred to a boiling water bath for 15 min.

After the samples were removed from the bath, 7.1 ml of distilled water was added to adjust the normality of the solution to 0.5. The contents were mixed and left at room temperature. Thirty minutes later, the fluorescence was read on a spectrophotofluorometer[3] with the emission monochromator rotary slit at 5, the excitation wavelength at 275 nm, and the emission wavelength at 450 nm (uncorrected). Tissue blanks, using tissues of untreated animals, and a set of standards were run simultaneously with each set of unknowns.

[1] Model UV, International Equipment Co.
[2] Eberbach model S1103.
[3] Model SPF, American Instrument Co.

0022-3549/79/0900-1097$01.00/0