Table II—Hypolipidemic Activity of 3,4,5,6-Dibenzohomopiperidine in Male CF$_1$ Mice [a]

Dose, mg/kg/d	Serum Cholesterol		Serum Triglyceride
	Day 9	Day 16	Day 14
Control (1% Carboxymethylcellulose)	100 ± 5[c]	100 ± 6[d]	100 ± 6[e]
Compound XVIII			
10	61 ± 5[b]	50 ± 5[b]	48 ± 5[b]
20	68 ± 4[b]	48 ± 4[b]	49 ± 5[b]
40	67 ± 6[b]	54 ± 4[b]	44 ± 6[b]
60	69 ± 5[b]	51 ± 4[b]	55 ± 6[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p < 0.001$. [c] 125 mg%. [d] 122 mg%. [e] 137 mg/dL.

less active than I in both the cholesterol and triglyceride screens; interestingly, the completely reduced compound isoindoline (III) was more active than the partially reduced phthalimidine (II) in both screens. In the succinimide series, the reduced compounds VII and VIII demonstrated less hypotriglyceridemic activity; the partially reduced compound 2-pyrolidinone (VII) demonstrated less hypocholesterolemic activity, but the totally reduced compound, pyrrolidine (VIII) was more active than succinimide in the cholesterol screen, causing 31% reduction. In the glutarimide series, the partially reduced compound, valerolactam (XII), demonstrated approximately the same activity in the cholesterol screen as glutarimide, but it demonstrated less hypotriglyceridemic activity than glutarimide. The fully reduced compound, piperidine (XIII) was more effective in both screens, with a marked reduction, 66%, of serum triglyceride levels. The reduction of diphenimide led to improved hypocholesterolemic activity for both derivatives, lowering serum cholesterol levels 51-49% and the fully reduced derivative serum triglyceride levels 48%. In the adipimide series, the reduction of the carbonyl groups led to little change in hypolipidemic effects in either screen.

Examination of the hydrolytic products of the phthalimide series showed that the half amide, phthalamic acid (IV), afforded approximately the same hypocholesterolemic activity as phthalimide; however, IV produced less effect on lowering triglyceride levels than I. The phthalic acid (V) demonstrated less activity in both screens compared to I. The hydrolytic products of the succinimide series (IX and X), the glutarimide series (XIV and XV), and the adipimide series (X, XXIV, and XXV) demonstrated no improvement in hypolipidemic activity over the parent derivatives. In the diphenimide series, the hydrolytic products XIX and XX demonstrated approximately the same hypocholesterolemic activity as XVI.

In conclusion, there did not appear to be a trend among all of the reduced products of imide analogues with respect to improvement or loss of activity in the hypolipidemic screens in mice. In general, the new compounds examined in this study did not extensively improve the hypocholesterolemic and hypotriglyceridemic activity of the lead compound, phthalimide (I). Piperidine produced improved hypotriglyceridemic activity compared to I, and XVII demonstrated improved hypocholesterolemic activity compared to I. The compound which demonstrated the best activity in both screens, other than I, was XVIII, which was slightly more potent than I in the cholesterolemic screen (6%) and slightly less active in the triglyceridemic screen (8%) in mice (Table II). These differences are probably not significant. Thus, I and XVIII are very similar in hypolipidemic activity. A dose response study with XVIII from 10-60 mg/kg showed that a 20 mg/kg/d dose was optimum for reducing serum cholesterol levels, and a 40 mg/kg/d dose lowered serum triglyceride levels to the level observed for I at 20 mg/kg. Further investigation of these derivatives is warranted since they are more active than commercially available agents, *e.g.*, clofibrate.

REFERENCES

(1) I. H. Hall, P. J. Voorstad, J. M. Chapman, Jr. and G. H. Cocolas, *J. Pharm. Sci.* **72,** 845 (1983).

(2) J. Bornstein and J. E. Shields, *Org. Synth.*, **Coll. Vol. V,** 1064 (1973).

(3) E. Chapman and H. Stephen, *J. Chem. Soc.*, **127,** 1791 (1925).

(4) J. H. Maguire and K. H. Dudley, *Anal. Chem.*, **49,** 292 (1977).

(5) H. W. Underwood and E. L. Kochmann, *J. Am. Chem. Soc.*, **46,** 2072 (1924).

(6) J. M. Chapman, Jr., G. H. Cocolas and I. H. Hall, *J. Med. Chem.* **28,** 243 (1983).

(7) A. Uffer and E. Schlettler, *Helv. Chim. Acta*, **31,** 1397 (1948).

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Liquid Crystals as a Potential Ointment Vehicle

SVEN WAHLGREN *, ANNA L. LINDSTROM ‡, and STIG E. FRIBERG §x

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Abstract □ A lecithin-water lyotropic liquid crystal was used as an ointment vehicle for a hydrocortisone formulation. The hydrocortisone was soluble in the liquid crystalline phase up to 5% by weight. The diffusion coefficient determined for the hydrocortisone in the liquid crystalline phase was 5.5×10^{-9} cm·s^{-1}, which is four magnitudes higher than the corresponding value for skin.

Keyphrases □ Ointments—vehicles, liquid crystals, lecithin-water □ Lecithin—liquid crystals as potential ointment vehicle □ Hydrocortisone—lecithin-water liquid crystals as potential ointment vehicle

The total therapeutic effect of percutaneous preparations depends not only on the action of the drug itself, but also on other factors related to the structure of the vehicle (1, 2). These latter factors may be divided into two main groups.

The first group contains vehicle-barrier interactions, mostly involving changes in the structure of the stratum corneum caused by the vehicle. These interactions may be evaluated *in toto* by use of standardized tests such as the blanching test (3, 4). Stratum corneum structural changes may facilitate or retard the diffusion of the active substance through this layer, as found for the absorption of solvents through skin (5). It has been claimed that some substances enhance penetration of pharmacological agents when topically applied (6).

The second group includes vehicle-drug interactions. Of these, the capacity of the structure to dissolve (solubilize) the active substance, the related chemical potential difference of the drug in the vehicle and in the stratum corneum, and the diffusion rate of the drug through the vehicle are the most important (7). The vehicle is usually a liquid that is immobilized by the presence of polymers or solid particles (1). The factors mentioned above are, therefore, related to the properties of the liquid phase; the immobilization of the bulk has little importance for the drug diffusion.

The possibilities of using colloidal structures as ointment

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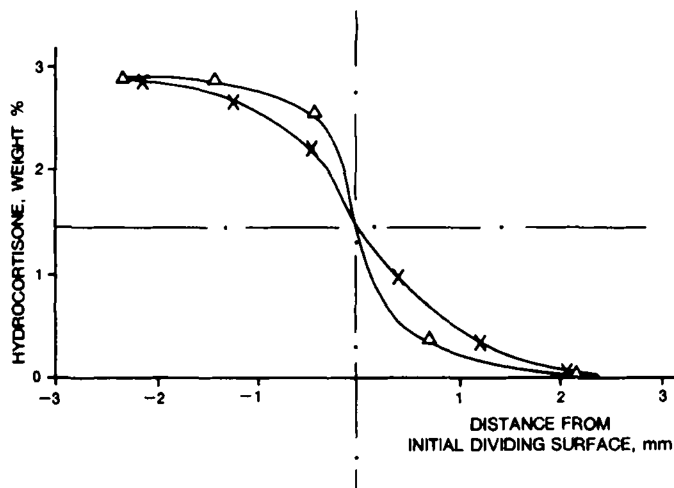

Figure 1—*Concentration profile of hydrocortisone as a function of distance from the initial dividing interface at different times after the start of the experiment. Key: (△) 96 h; (X) 192 h; (---) t = ∞.*

vehicles in a systematic manner have not been utilized in preparations so far, in spite of the interesting solubility properties of these structures (8). This report describes the properties of a lamellar liquid crystal for use as the basic vehicle for an ointment preparation.

The preparation appears to have a series of advantages. It is thermodynamically stable, the solubility in hydrocortisone is considerably higher than in a liquid, and the diffusion of the active substance is higher than is the case for a suspension of solid hydrocortisone, the common preparation form.

EXPERIMENTAL SECTION

Materials—Micronized hydrocortisone[1], and a soy lecithin[2] with 95% phosphatidylcholine were obtained commercially. The purity was checked by TLC. The water was twice distilled.

Preparation—The liquid crystals were prepared by the general technique introduced by Loomis *et al.* (9). The hydrocortisone was dissolved to 1.5% by weight in absolute alcohol, and the lecithin was dissolved to 10% in chloroform. Different volumes of the two solutions to give required hydrocortisone-lecithin ratios were mixed, and the solvent was evaporated at ambient temperature and reduced pressure. The range of volumes were 1-1.95 to give 5% by weight of hydrocortisone in the liquid crystal. Water in a 3:7 weight ratio was added to the hydrocortisone-lecithin mixture and homogenized by a combination of heating to 50°C and vibrational stirring.

The maximum solubility of hydrocortisone was directly detected by visual observation in a microscope with polarized light against the liquid crystalline background. A series of samples with a different percentage of hydrocortisone were examined, and the solubility limit was determined to be the highest hydrocortisone concentration at which no crystals could be observed. The hydrocortisone crystals are a conspicuous feature and easily detected. The presence of crystals was verified from their X-ray diffraction patterns with a camera[3].

Structure Determinations—The liquid crystalline structure was identified from its optical pattern in polarized light, and the spacing ratios in the low-angle X-ray diffraction pattern were obtained with a camera[4].

Diffusion Coefficient—The diffusion rate was determined in the following manner. A glass cylinder (23 mm i.d.) was filled with liquid crystal-containing hydrocortisone. An identical cylinder with the liquid crystal without hydrocortisone was placed on top of the first one with the free surfaces in complete contact. The cylinders were dismantled at different times, and the liquid crystal was pressed from the cylinders, scraped off, and weighed to enable exact calculations of the thickness of removed layers. The cortisone concentration was determined photometrically according to Pharm. Nord. (see *Appendix*).

[1] Upjohn S.A., Lichterstraat, B 2670, Puurs, Belgium.
[2] Epikuron 200; Lucas Meyer, Ausschläger, Elbdeich 21, Hamburg 28, Federal Republic of Germany.
[3] Debye-Scherrer; Seifert & Co., Hamburg, Federal Republic of Germany.
[4] Kiessig; Seifert & Co.

RESULTS

Solubility of Cortisone—The maximum solubility of hydrocortisone in the liquid crystal with 70% lecithin-30% water was 5.0% by weight. This meant a molecular ratio of 1:6 for hydrocortisone-lecithin.

Low-Angle Diffraction—The repeat fraction distance showed a 1:2 spacing ratio, confirming the optical patterns which indicated a lamellar structure. The interlayer spacing for the sample without hydrocortisone was calculated to be 5.3 nm, which is in good agreement with previously reported values (10). A sample with 3% hydrocortisone gave exactly the same interlayer spacing.

Diffusion Rate—The concentration of hydrocortisone *versus* the distance at 96 and 192 h is shown in Fig. 1. The curves show excellent symmetry across the separating surface, which is marked as distance zero on the diagram.

Calculation of Diffusion Coefficient—The second equation of Fick states that:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{dx^2} \quad \text{(Eq. 1)}$$

where C is the concentration of diffusing substance, t is time, D is the diffusion coefficient, which is assumed to be constant, and x is the distance along the diffusion axis. From this relation, an explicit expression can be given for the relation between concentration, time, and coordinate for the system in question:

$$C = \frac{C_0^1}{2}[1 - \psi(x/2\sqrt{Dt})] \quad \text{(Eq. 2)}$$

where $\psi(x/2\sqrt{Dt})$ is the probability integral. The diffusion coefficient is obtained by:

$$D = \frac{x^2}{4t}\left[\frac{1}{\psi^*\left(1 - \frac{2C}{C_0^1}\right)}\right]^2 \quad \text{(Eq. 3)}$$

where $\psi^*(1 - 2C/C_0^1)$ is the inverse function of the probability integral.

The calculations were made by using the values shown in Fig. 1. The parameter $1 - 2C/C_0^1$ was calculated for a series of points along the curves, and numerical integration was used to obtain $\psi^*(1 - 2C/C_0^1)$. The values obtained gave an average of $D = 5.0 \times 10^{-9} \pm 0.1 \times 10^{-9}$ cm·s^{-1} with a 95% confidence interval.

DISCUSSION

The results of this study show two features of importance for the use of a liquid crystal as an ointment vehicle for percutaneous hydrocortisone preparations. The first feature is the high solubility of hydrocortisone in the lamellar liquid crystalline phase. The value is about four times greater than the corresponding solubility of hydrocortisone in the commonly used ethylene glycol. The reason for this high solubility is not the subject of this report, but the qualitative information may be useful for other applications. According to Israelachvili *et al.* (11), the free energy of a planar layer in a liquid crystal may not be the lowest energy level of an amphiphile-water system. A vesicle with a certain radius may be a more stable configuration for a certain volume of the hydrocarbon chains and a certain cross-sectional area of the polar group. The ratio between the two free energies is $(1 - \alpha)$, in which α depends on the factors mentioned above.

This condition means that the addition of a solute to the structure may in some cases lower the structure-dependent part of the free energy and hence give a higher solubility than can be achieved in an isotropic liquid with a similar chemical composition.

The second feature of importance is the large value of the diffusion coefficient. Its value is distinctly in excess of the one determined for passage through skin. This fact implies a full benefit of the high concentration in the liquid crystal during percutaneous treatment with hydrocortisone. The barrier giving rise to slow release in the commonly used dispersion does not exist in a liquid crystal vehicle. This high diffusion coefficient is related to the location of the hydrocortisone in the structure of the lamellar liquid crystal, and a discussion of the site of the hydrocortisone molecules may be of interest.

The low-angle X-ray diffraction studies showed identical values for the liquid crystal with and without hydrocortisone. This indicates that the hydrocortisone molecules are located between the lecithin molecules in the liquid crystalline structure. A location between the layers would have given a proportional increase of the interlayer spacing. A location between the molecules would facilitate the diffusion of the hydrocortisone molecules.

Such a location and the structure of hydrocortisone make a comparison meaningful with the lecithin-cholesterol-water system (12), which has been

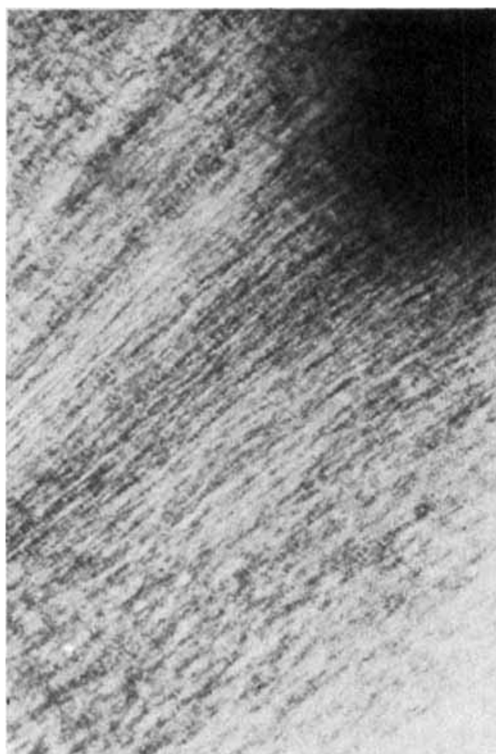

Figure 2—*The optical pattern in polarized light typical of a lamellar structure.*

carefully investigated with regard to its importance for atherosclerotic lesions (13). The influence on the chain order by the presence of cholesterol has been evaluated by McIntosh (14) for the gel state. In all cases the cholesterol molecule is located between the lecithin molecules, and its influence on the interlayer spacing is ascribed to change in tilt and/or order of the hydrocarbon chains. It appears reasonable to assume that the location of the hydrocortisone molecule is between the lecithin molecules and that its diffusion mainly takes place within the constraints of the hydrophobic parts of the lamellar structure.

Diffusion coefficients for the lecithin molecules in liquid crystals with cholesterol have recently been determined by pulsed-spin echo NMR (15). The presence of cholesterol up to 33 mol % counted on lecithin increased the diffusion coefficient for the lecithin by 33%. The amounts of hydrocortisone present in the liquid crystal investigated were less than half the value in the case of cholesterol, and it appears that the presence of hydrocortisone has a relatively minor influence on the diffusion conditions.

The value found for the diffusion coefficient of hydrocortisone was ~10 times less than the one determined previously for lecithin (15) in a lamellar liquid crystal. Two factors contribute to the difference. The values found for lecithin are for the local diffusion parallel to the layers. The values reported for hydrocortisone are bulk volumes for a body of the liquid crystal in which layers have orientations varying in all directions.

A completely statistical distribution of diffusion directions in space would reduce the diffusion coefficient. By assuming equal distribution in all directions and neglecting diffusing perpendicular to the layer plane, the ratio between diffusion as measured by NMR (D_{NMR}) and the total diffusion coefficient measured in this investigation (D_{tot}) would be the average over all angles:

$$D_{tot}/D_{NMR} = \frac{2}{\pi}\int_0^{\pi/2} \sin\theta d\theta = 2/\pi \qquad \text{(Eq. 4)}$$

The ratio between measured values is obviously larger than this, and additional factors must be found to explain the difference.

One such factor originates from the dislocation pattern in a lamellar phase (16). The dislocations are mainly of the focal-conical type or other closed figures. Any part of the total space in the sample in which the layers form a closed figure adds to the total diffusion only to an insignificant amount. A superficial observation of a typical dislocation pattern of a lamellar liquid crystal in this concentration range (Fig. 2) does not rule out a considerable part of the structure consisting of closed figures. The overall diffusion coefficient found in this manner appears to be of reasonable magnitude for a system in which the hydrocortisone molecules are located between the lecithin molecules that diffuse in the hydrophobic layers only. The diffusion coefficient for hydrocortisone in a lamellar liquid crystal was considerably higher than the one for passage through skin; this is a factor of importance for some topical dosage forms.

APPENDIX

Reagents—The following reagents were used for hydrocortisone analysis: absolute ethanol; concentrated acetic acid; a 10% solution of tetramethyl ammonium hydroxide (TMAH)[5], which was kept refrigerated in the dark; 1% TMAH by dilution of 10% TMAH with 9 parts ethanol; 2,3,5-triphenyl tetrazolium chloride (TPTC)[6], which was kept in the refrigerator and dark; 0.5% of TPTC in ethanol, which was prepared immediately before use and protected against light; hydrocortisone standard (50 mg of hydrocortisone standard was dissolved in 100 ml of ethanol; 10.00 mL of this solution was diluted to 100.00 mL with ethanol).

Procedure—Ointment (0.2500 g) was weighed in a 50-mL flask. Ethanol (40 mL) was added, and the mixture was heated to boiling on a water bath with careful stirring. The flask was removed from the water bath and shaken vigorously.

After cooling to room temperature, alcohol was added to 50 mL and the solution was filtered; 5 mL of filtrate and 13 mL of ethanol were added to a 25-mL flask with 2.0 mL of 1% TMAH, and after careful shaking with 2.0 mL of TPTC, 0.5% TPTC was carefully added.

The solution was stored in the dark for 60 min and after 0.05 mL of concentrated acetic acid and ethanol to 25 mL were added, the extinction was determined at 485 nm in a 1-cm cell. As the reference solution was used, 18 mL of ethanol was treated analogously. Calibration was made by using an identical procedure on 5 mL of the hydrocortisone standard and 13 mL of ethanol.

REFERENCES

(1) H. Junginger, H. Heering, C. Führer, and I. Geffers, *Colloid Polym. Sci.*, **259**, 561 (1981).

(2) B. C. Lippold and A. Teubner, *Pharm. Ind.*, **43**, 1123 (1981).

(3) R. Woodford and B. W. Barry, *J. Pharm. Sci.*, **66**, 99 (1977).

(4) B. W. Barry and R. Woodford, *Br. J. Dermatol.*, **97**, 555 (1977).

(5) I. Jacobson, B. Holmberg, and J. E. Wahlberg, *Acta Pharmacol. Toxicol.*, **41**, 497 (1977).

(6) R. B. Stoughton, *Arch. Dermatol.*, **118**, 474 (1982).

(7) H. Schaefer, B. Schroot, and W. Schalla, "Symposium Abstracts Topical Administration of Drugs," Swedish Academy of Pharmaceutical Sciences, 1983, p. 21.

(8) P. Ekwall, in "Advances in Liquid Crystals," vol. 1, G. H. Brown, Ed., Academic, New York, N.Y., 1975, p. 1.

(9) C. R. Loomis, M. J. Janiak, D. M. Small, and G. G. Shipley, *J. Math. Biol.*, **86**, 309 (1974).

(10) N. Moucharafieh and S. E. Friberg, *Mol. Cryst. Liq. Cryst.*, **49**, 201 (1979).

(11) J. N. Israelachvili, D. J. Mitchell, and B. W. Ninham, *J. Chem. Soc. Faraday Trans. II*, **72**, 1525 (1976).

(12) M. Bourgès, D. M. Small, and D. G. Dervichian, *Biochim. Biophys. Acta*, **137**, 157 (1957).

(13) D. M. Small and G. G. Shipley, *Science*, **185**, 222 (1974).

(14) T. J. McIntosh, *Biochim. Biophys. Acta*, **513**, 43 (1978).

(15) G. Lindblom, *Acta Chem. Scand. Sect. B*, **35**, 61 (1981).

(16) M. Kleman, C. E. Williams, M. J. Costello, and T. Gulik-Kozyniski. *Phil. Mag*, **35**, 33 (1977).

[5] Merck 8123.
[6] Merck 8380.