# *In Vitro* Cutaneous and Percutaneous Delivery and *in Vivo* Efficacy of Tetracaine from Liposomal and Conventional Vehicles

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The aim of this study was to quantitate drug delivery and correlate drug concentration in the skin with anesthetic effect. The rate of delivery of radiolabelled tetracaine from two different liposome formulas (1F2 and 23C2) and two conventional dosage forms (PEG Ointment USP and Glaxal® base) was investigated in Flow-thru diffusion cells using human breast skin from mammoplasty. The results indicated a 1.5 and 4 times higher concentration of tetracaine within the skin when the liposomal formula (1F2) was used, compared to tetracaine in Glaxal® base and PEG Ointment USP, respectively. The amount of drug delivered into the skin in 24 h from the liposomal formula was 5.3% of total applied whereas from Glaxal® base it was 3.3% and from PEG Ointment base it was 1.2%. The amount of liposomal (1F2) phospholipids in the skin after 24h was 68.3 $\mu g/cm^2$ (0.2% of total applied). The steady state flux of tetracaine from liposomes (1F2) was 16.06 $\mu g/cm^2/h$ with a lag time of 3.1h, from Glaxal® base 10.24 $\mu g/cm/h$ with a lag time of 11.2h and from PEG Ointment it was 5.70 $\mu g/cm^2/h$ with a lag time of 9.0h. The second liposome formula (23C2) showed similar flux and permeability coefficient than the Glaxal® base, however the lag time was about half. The results indicated that optimized liposome formulation is necessary to achieve maximum drug delivery. The concentration of drug within the skin and the flux measured *in vitro* showed correlation with *in vivo* efficacy. The *in vivo* data showed that liposomal (1F2) tetracaine produced the deepest anesthesia with shortest onset in volunteers, followed by Glaxal® base, liposome formula 23C2 while tetracaine in PEG Ointment had a lack of effect.

KEY WORDS: liposomes; topical dosage forms; local anesthetics; cutaneous and percutaneous absorption.

## INTRODUCTION

The need for the development of an effective topical anesthetic preparation prompted researchers to try various approaches. Earlier experiments by Dalili and Adriani (1) showed the lack of efficacy of most local anesthetic drugs in topical vehicles, because of the lack of penetration (delivery) of sufficient amount of drug to the target site i.e. the dermally located sensory nerves. The development of EMLA cream (eutectic mixture of local anesthetics; lidocaine and prilocaine) was a step forward in the achievement of a more efficient topical anesthetic effect. Several studies indicated that upon application of a 'thick layer' of EMLA cream for at least 2h, a reasonable effect develops (0.5 mm deep anesthesia) in adults and in children (2-3).

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Woolfson and coworkers (4) showed topical anesthetic effect using a high (4%) concentation of tetracaine in Carbomer gel at 0.5 g preparation/site for 30 min in human subjects. The onset of effect was detectable by the pin-prick method at 40 min and it lasted for 2-3 hours. Further clinical studies demonstrated a fairly good success (70.8% reported no pain) in venepuncture with the 4% tetracaine gel product (5). Comparative studies indicated that 4% tetracaine gel produced more rapid and prolonged anesthesia than EMLA (5).

Recently Planas et al (6) reported the use of 'transferosomes' a surfactant/lipid vesicle system (an admittedly unstable preparation) for topical treatment with lidocaine (7%) and tetracaine (4%). After a single application on human forearms the analgesic effect only lasted for about 80 min and 30 min for the lidocaine and tetracaine preparation, respectively. The degree of anesthesia, which was determined by the pin-prick method, was moderate (authors accepted 6 painless scores out of ten as evidence of anesthesia). Prolonged anesthesia was only achieved by repeated applications.

The above studies all indicate that the achievement of significant (rapid, deep and long lasting) skin anesthesia is a challenging problem.

Encouraging progress in this area was made by Mezei and Gesztes (7), who reported the use of liposome encapsulated tetracaine (0.5%) for topical anesthesia in volunteers. These results demonstrated that by liposomal delivery significant skin anesthesia (as assessed by the pin-prick method) can be achieved using low concentration of tetracaine (8). Randomized, double blind clinical experiments in 150 patients undergoing venepuncture showed that the 0.5% liposomal tetracaine product was significantly different from placebo and there was no difference in the incidence of erythema, edema or blistering in the two test groups (9). Although the liposomal tetracaine (0.5%) versus placebo control was statistically significant, the magnitude of the actual pain difference appeared to be small indicating that during needling procedures a deeper anesthesia is required beyond that of the pin-prick method.

Subsequently to the above studies we reported the design and partial evaluation of topical dosage forms of liposomal tetracaine (2%) to improve the rate of penetration into the skin and develop a more effective formula suitable for relieving pain in venepuncture, lumbar puncture and other minor surgical procedures involving the skin (10, 11).

In this paper results of *in vitro* cutaneous and percutaneous absorption of tetracaine into and through human breast skin from liposomal and conventional topical creams was compared and correlated with topical anesthetic effect *in vivo*.

In order to accept and use liposomes as a new dermatological dosage form more information is necessary on the bioavailability and bioequivalency of liposome encapsulated drugs. The main purpose of this investigation was to characterize the absorption of tetracaine from representative topical liposomal and conventional vehicles, in order to gain more detailed understanding of the mechanism of liposomal drug delivery to the skin.



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## MATERIALS AND METHODS

### Materials

Soya phosphatidylcholine, hydrogenated (Phospholipon 90H) was obtained from Natterman Phospholipide GmbH, Cologne, Germany. Cholesterol, tetracaine base and butylhydroxyanisole (BHA) were from Sigma Chemical Company, St Louis, MO. Stearic acid USP grade was obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. Crude tritiated tetracaine was obtained from Amersham Canada Ltd. (Oakville, On.) and purified by thin layer chromatography in our laboratory (specific activity: 3.61 mCi/mg). L-3-phosphatidyl [N-methyl-$^{14}$C] choline, 1,2-dipalmitoyl (DPPC) (specific activity: 58 mCi/mMol) was obtained from Dupont Canada Ltd. (Mississauga, On.).

### Preparation of Liposomes

Multilamellar liposomes were prepared by the solvent evaporation method (12). The lipid phase was first dissolved in chloroform: methanol (2:1) in a round bottom flask containing glass beads. The solvent was then removed by rotary evaporation at 25-30°C (Buchi RE 111 Rotavapor, Buchi Laboratoriums, AG Flawil/Sheiz, Switzerland) such that a thin lipid film was deposited on the wall of the flask and the surface of the beads. Once the thin lipid film was formed the flask was heated to the gel-to-liquid crystalline transition temperature of the lipids (approx. 55°C). The appropriate aqueous phase was then added and the flask hand-shaken vigorously for two minutes to hydrate the lipid film. The flask was then placed into a waterbath at 55°C and shaken for 20 minutes continuously to complete liposome formation (Girotory waterbath shaker, model G76, New Brunswick Scientific Co. Inc., New Brunswick N.J.).

Composition of liposomal and conventional formulations (% w/w): Liposome **1F2**: Phospholipon 90H 10%, cholesterol 1.75%, stearic acid 0.7%, tetracaine 2%, Aqueous phase F: NaCl 0.9, Propylene glycol 7.0, Ethanol 10.0, BHA 0.02, pH 5.5; Liposome **23C2**: Phospholipon 90H 10%, Cholesterol 1.75%, Stearic acid 1.75%, Tetracaine 2%, Aqueous phase C: NaCl 0.45, $NaHCO_3$ 0.65, Propylene glycol 15.0, Ethanol 10.0, BHA 0.02, pH 8.5; **Glaxal base**: Glaxal® base, 2% Tetracaine; **PEG Ointment**: PEG Ointment USP, 2% Tetracaine. Each preparation contained 15μCi $^3$H-tetracaine/g product.

### Percutaneous Absorption Studies

The rate of diffusion of tetracaine from the liposome preparations across full thickness human breast skin from plastic surgery was investigated using Teflon® Flow-Thru Diffusion Cells (Crown Glass Co. Inc. Somerville N.J.) which have a surface area for diffusion of 0.32 cm$^2$ (13). The diffusion cells are designed such that fluid may be continuously pumped through them in order to maintain sink conditions. A phosphate buffer (7.5 mM $Na_2HPO_4$, 2.5 mM $NaH_2PO_4$ 141.2 mM NaCl) isotonic with body fluids and having a pH of 7.2 was used as the perfusion fluid.

The diffusion cells were mounted in a PosiBloc™ Diffusion Cell Heater (Crown Glass Co. Inc. Somerville N.J.), maintained at 32°C by a circulating water bath. The flow rate was 3 mL per hour. Each experiment was conducted for a period of 24 hours continuously. Liposome preparation containing 2 mg of tetracaine (0.1 g preparation), dual labelled with $^{14}$C-DPPC and $^3$H-tetracaine, was instilled into each cell at the beginning of each experiment. Three replicates of each experiment were performed. The quantity of tetracaine in each sample was determined by liquid scintillation counting.

### Penetration of Liposome Encapsulated Drug into Human Breast Skin

At the end of the 24 h experiment the skin was removed from the diffusion cell, rinsed with PBS buffer (3 × 5mL) and sectioned with a cryostat. The serial sections were digested with NCS tissue solubilizer, neutralized and the drug concentration determined by scintillation counting.

### Data Analysis

Data are plotted as cumulative amount of drug diffused (Q) as a function of time (t). The permeability coefficient (P) was calculated from Fick's first law:

$$(dQ/dt)_{SS} = J_{SS} = P\,\Delta C \qquad \text{where } P = Kp\,D/h$$

where

$J_{SS}$ steady state flux (calculated by regression analysis of the linear portion of the curve)

$\Delta C$ concentration difference between donor and receiver compartments

Kp partition coefficient between skin and the preparation

Diffusion coefficient was calculated from

$$D = h^2/6L$$

where

h thickness of the stratum corneum (0.001 cm)

L lagtime (sec)

### Testing the Efficacy of Topical Liposomal Tetracaine Preparations in Volunteers

Eleven volunteers have participated in this part of the study following their written consent. In a double-blind design five preparations (liposomal placebo, formulas: 23C2, 1F2, "commercial" controls: 2% tetracaine in PEG Ointment USP and Glaxal base) were randomly numbered by a person not participating in the experiments. A single dose of 0.2g of the above preparations was applied on five premarked 10 cm$^2$ area on the forearm of the volunteers in a random fashion. The treated area was covered with parafilm and Tegaderm® dressing (3M Co, St. Paul, Minnesota) to provide occlusion for 15 min. After 15 min the preparations were removed from the skin by wiping with a tissue. The pin-prick test was used to assess the local anesthetic effect (14). The skin was pricked with a relatively blunt needle ten times and the number of painful pricks were recorded. Testing was done immediately after removal of the sample and at 15, 30, 45, 60, 90, 120 and 240 minutes. The preparations were compared by paired t-tests.

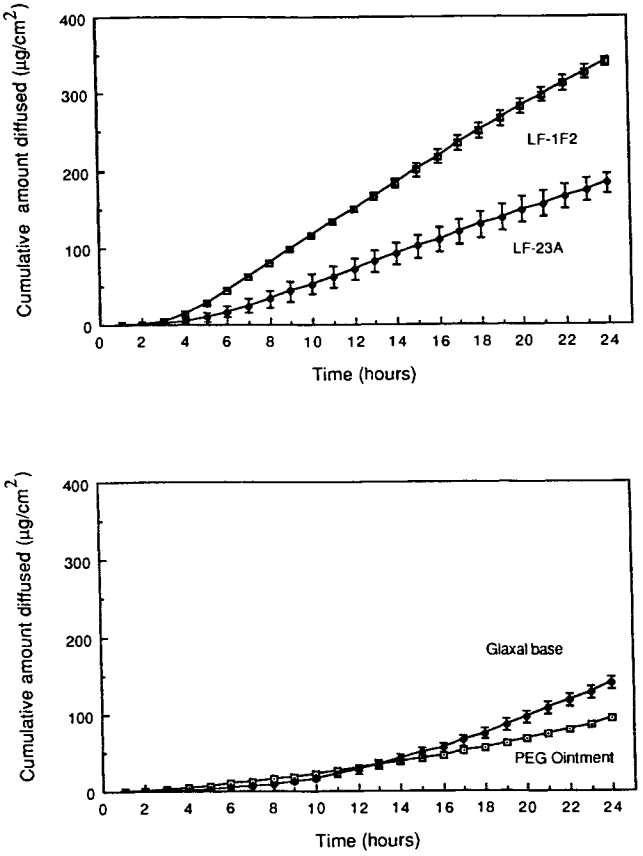


**Fig 1** *In vitro* percutaneous absorption of tetracaine from topical liposomal and conventional vehicles using full thickness human breast skin. The cumulative flux (μg/cm²/h) of tetracaine is shown for liposomal formulas (LF) 1F2 and 23C2 (Panel A) and conventional formulas Glaxal base and PEG Ointment (Panel B). Each point represents the mean ±S.D. n=3 separate experiments.

## RESULTS

### *In Vitro* Absorption of Tetracaine into and Through Breast Skin

The *in vitro* percutaneous absorption experiments indicated that significantly larger amounts of tetracaine were delivered through the skin from the liposomal formulas than from either conventional preparations (Fig 1). The absorption of tetracaine was, however, also different from the two liposomal formulas. Liposome formula 1F2 showed a 1.8 times higher steady state flux value (16.06 μg/cm²/h) compared to liposome formula 23C2 (8.65 mg/cm²/h). While tetracaine flux ($J_{ss}$) from Glaxal base (10.24 μg/cm²/h) was similar to liposome formula 23C2. In case of PEG Ointment only 5.70 μg/cm²/h flux ($J_{ss}$) could be achieved (Table 1).

The total amount of drug diffused through the skin within 24h was the highest for liposome formula 1F2, 341.13±6.02 μg/cm², followed by liposome formula 23C2 (182.49±13.30 μg/cm²), Glaxal base (139.49±7.60 μg/cm²) and PEG Ointment (92.91±11.21 μg/cm²) (Table 1). The lag time for the appearance of tetracaine in the receiver compartments was significantly lower for the liposome formulas (3.1 and 4.2 h versus 11.2 and 9.0 h, see Table 1).

After the diffusion cell experiment the skin was thoroughly washed and sectioned with a cryostat to determine the distribution of tetracaine within the skin. The cutaneous absorption of tetracaine was 1.5-4 times higher from liposome formula 1F2 than from the other conventional vehicles (Table 2). This difference was maintained throughout the epidermis and dermis (Fig 2). Interestingly Glaxal base provided higher (257.6 μg/cm²) tetracaine levels in the skin than the liposome formula 23C2 (126.9 μg/cm²) (Table 2). The amount of liposomal phospholipids absorbed into the skin was proportionally increasing with increasing drug concentration (Table 2). From liposome formula 1F2 369.9 μg/cm² tetracaine (5.35% of total applied) and 68.3 μg/cm² phospholipid penetrated the skin (0.20% of total), whereas from liposome formula 23C2 126.9 μg/cm² (1.7% of total) and 22.5 μg/cm² was detected in the skin (0.06% of total). The distribution of phospholipids within the skin appeared to follow the drug distribution (Fig 2B), however, the lipid/drug ratios in the skin were different than that in the original liposome preparation.

### Topical Anesthetic Effect in Volunteers

Experiments in volunteers indicated significant differences in the topical anesthetic effect of the various formulas. In Fig 3 the mean painful scores out of 10 pricks on the skin treated with placebo or one of the tetracaine formulations versus time were plotted. After 15 min application time tetracaine in PEG Ointment had no effect. The painscores were similar to the placebo preparation (Fig 3). Liposome formula 1F2 and Glaxal base showed similarly effective topical anesthesia, i.e. pain score of 3-4 at 30 min, after skin treatment for 15 min, ($p>0.4$).

Liposome formula 23C2 produced a somewhat weaker anesthetic effect on the skin at earlier time intervals. With this latter formula sufficient anesthesia (pain score lower than 2) could be achieved only after 3h (Fig 3). The difference between liposome formulas 1F2 and 23C2 or Glaxal base and liposome 23C2 were statistically significant ($0.0005 \leq p \leq 0.005$). Both liposome preparations and Glaxal base were significantly better than PEG Ointment and placebo ($p \leq 0.0005$). There was no significant difference between liposome 1F2 and Glaxal base.

**Table I.** *In Vitro* Absorption of Tetracaine from Liposomal and Conventional Vehicles Through Breast Skin

| Preparation | Amount diffused in 24 h (μg/cm²) n = 3) | Steady State Flux (μg/cm²/h) | Permeability coefficient (cm/h) | Diffusion coefficient (cm²/sec) | Lag time (h) |
|---|---|---|---|---|---|
| Liposome 1F2 | 341.13 ± 6.02 | 16.06 | $8.03 \times 10^{-4}$ | $8.96 \times 10^{-11}$ | 3.1 |
| Liposome 23C2 | 182.49 ± 13.30 | 8.65 | $4.32 \times 10^{-4}$ | $6.61 \times 10^{-11}$ | 4.2 |
| Glaxal Base | 139.49 ± 7.60 | 10.24 | $5.12 \times 10^{-4}$ | $2.48 \times 10^{-11}$ | 11.2 |
| PEG Ointment | 92.91 ± 11.21 | 5.70 | $2.85 \times 10^{-4}$ | $3.09 \times 10^{-11}$ | 9.0 |

**Table II.** Cutaneous Absorption of Tetracaine and Phospholipids into Breast Skin Treated with Liposomal and Conventional Vehicles at Steady State

| | $^3$H-tetracaine | | $^{14}$C-DPPC* | |
|---|---|---|---|---|
| Preparation | μg/cm$^2$ | % of total | μg/cm$^2$ | % of total |
| Liposome 1F2 | 369.9 | 5.3 | 68.3 | 0.20 |
| Liposome 23C2 | 126.9 | 1.7 | 22.5 | 0.06 |
| Glaxal Base | 257.6 | 3.3 | — | — |
| PEG Ointment | 92.9 | 1.2 | — | — |

* dipalmitoylphosphatidylcholine

After liposome treatment a longer duration of anesthetic effect was observed than with Glaxal base (8-14 h versus 5-8 h). Slight erythema could be observed at the site of application with all drug preparations, however, this effect was more pronounced with Glaxal base than with the liposomal formulas.

## DISCUSSION

To determine the effect of liposome encapsulation on the rate of absorption of tetracaine into and through the skin *in vitro* percutaneous absorption experiments were carried out.

Liposome encapsulation enhanced the cutaneous and percutaneous absorption of tetracaine compared to conventional ointments such as Glaxal base or PEG Ointment. The permeability coefficient ($K_p$) increased by 160-280% in liposomes (formula 1F2) compared to the two other vehicles examined. It is apparent from our data that there can be significant differences between liposomal formulas of different compositions. The $K_p$ with liposome formula 23C2 was actually lower than with Glaxal base. There was a significant difference between the steady state flux values for the different preparations (Table 1). The flux from a 2% gel preparation (8.65 μg/cm$^2$/h) reported by Woolfson et al. (15) appears to be similar to the conventional products studied in this paper, but it was two times lower than liposome formula 1F2. In the experiments by Woolfson et al. (15) the duration of the experiments, 7h, could have been too short to achieve steady state and this comparison might not represent the relative differences between their gel preparation and the products in this study. Unfortunately Woolfson et al., (15) did not report lag times or diffusion coefficients, and therefore, the data cannot be compared.

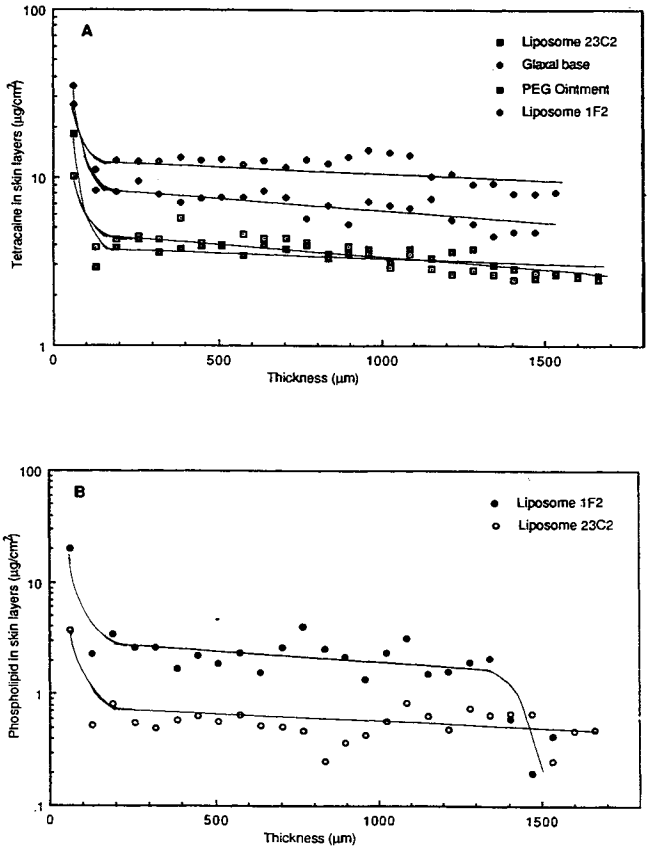


Fig 2 Cutaneous distribution of $^3$H-tetracaine (Panel A) and $^{14}$C-DPPC (Panel B) in breast skin treated with liposomal and conventional vehicles at steady state.

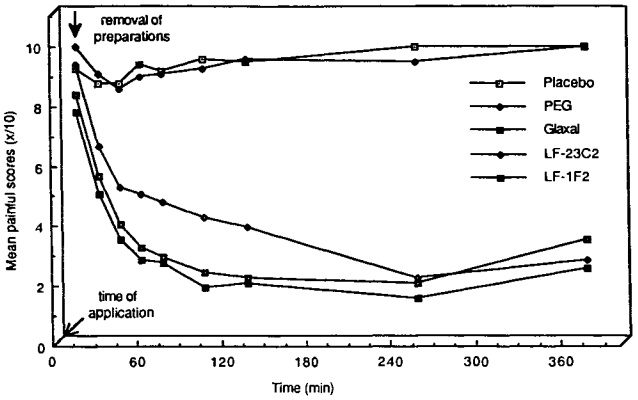


Fig 3 Assessment of topical anesthetic effect of liposomal and conventional formulas containing 2% tetracaine in volunteers. The placebo was an 'empty' liposome formula 1F2 with no encapsulated drug. Topical anesthetic effect was expressed as mean painful scores out of ten pinpricks (n = 16).

Lag times for the two liposome formulas (1F2 and 23C2) were 3.1 and 4.2 h, respectively, whereas for Glaxal base and PEG Ointment 11.2 and 9.0 h were observed, resulting in 3-5 times higher diffusion coefficient for the liposomal formulas.

The highest efficacy of liposome formula 1F2 in volunteers was consistent with the *in vitro* data. This formula had the shortest lag time and the highest flux. Similar results were obtained with Glaxal base in volunteers although this preparation had a 11.2 h lag time and about 1.6 times lower flux ($J_{ss}$). With liposome formula 23C2 the achievement of topical anesthesia was more gradual. This formula had lower flux than the liposome formula 1F2 and Glaxal base, but the lagtime was two times shorter than with Glaxal base; therefore, the poor performance of this preparation was unexpected. The difference in efficacy between the two liposomal formulations could be attributed to difference in pH of the preparations. In Formula 23C2 the pH (8.5) is around the pKa (8.39) of tetracaine therefore this preparation contains a higher proportion of unionized drug than liposome formula 1F2 (pH 5.5). Therefore it could be expected that from liposome formula 23C2 the drug penetration into the skin would be higher, but the opposite was found. Although the encap-

Table III. Hypothetical Calculations for Delivery of Tetracaine by Liposomes

| Preparation | Phospholipid detected in skin | Calculated number of liposomes** | Amt of tetracaine encapsulated in | Amt of tetracaine detected in skin liposomes** | Enhancement factor |
|---|---|---|---|---|---|
| Liposome 1F2 | 68.3 $\mu g/cm^2$ | $1.25 \times 10^9$ | 1.1 $\mu g$ ($2 \times 10^6$ tetracaine/ liposome) | 369.9 $\mu g/cm^2$ | 336 |
| Liposome 23A | 22.5 $\mu g/cm^2$ | $4.14 \times 10^8$ | 0.4 $\mu g$ ($2.2 \times 10^6$ tetracaine/ liposome) | 126.9 $\mu g/cm^2$ | 317 |

** calculated with the following assumptions: Liposomes are all MLV with 20 concentric bilayers and 0.5 $\mu m$ average size; 1 $\mu mol$ lipid = $2.5 \times 10^{10}$ vesicles

sulation efficiencies of the two liposomal formulas were similar (80.4% 1F2 and 86.4% 23C2 (10)), significant differences in the rate and extent of drug delivery could be observed. One explanation for these results could be that in the Formula 23C2, due to formation of sodium stearate from stearic acid and Na-bicarbonate (part of the aqueous phase) at high pH, the liposome structure may be slightly altered, the liposomes may be disrupted in the presence of this in situ formed anionic surfactant. The partial loss of liposomes may be detrimental for drug delivery. Tetracaine may be 'oversolubilized' in Formula 23C2.

Analysis of the drug concentration within the skin provided further explanation for the order of efficacy of these products. The efficacy of the products corresponds well with the amount of drug in the skin. The onset, depth and duration of local anesthetic effect of liposomal and conventional ointments in volunteers correlated well with the total amount of tetracaine absorbed. Liposome 1F2 provided the deepest and longest anesthetic effect, followed by Glaxal base and liposome formula 23C2. Tetracaine in PEG Ointment did not show any effect (Table 2). Fig 3 shows the distribution profile of tetracaine from the four preparations in breast skin. Tetracaine appears to be distributed fairly evenly throughout the viable epidermis and dermis in each case, however, the drug levels achieved are different. Highest drug levels were found with liposome formula 1F2 followed by Glaxal base, liposome formula 23C2 and PEG Ointment.

For the liposomal products the absorption of phospholipids (i.e., vehicle components) was also calculated to obtain more information about the interaction of the delivery system with the skin. A small but recognizable amount of liposomal phospholipid was associated with the skin (epidermis and dermis). Previous radiolabel and electron microscopic results (16, 17) indicated that liposomes may penetrate into the skin and deposit in the dermis. The frequency of this event, however, is still not completely determined. Table 3 shows a hypothetical consideration using the present experimental data. From the amount of phospholipids detected in the skin after treatment with liposome formula 1F2 or 23C2, the representative number of liposomes which could be formed was calculated. It was assumed that all liposomes were multilamellar with 20 concentric bilayers and had 0.5 $\mu m$ average size. It was assumed that 1 $\mu mol$ lipid can form $2.5 \times 10^{10}$ vesicles (18). From drug encapsulation data (liposome formula 1F2 had 80.4% encapsulation efficiency, and liposome formula 23C2 had 86.4%; (10)), the amount of tetracaine encapsulated in the number of liposomes associated with the skin could be calculated. Since in case of liposome formula 1F2 68.3$\mu g/cm^2$ phospholipid was found to be associated with the skin, this would correspond to $1.25 \times 10^9$ liposomes (for the sake of argument it is assumed that all the lipid in the skin was in the form of intact liposomes). These liposomes contain 1.1 $\mu g$ tetracaine i.e. $2 \times 10^6$ tetracaine molecules per liposome. This would mean that if delivery of tetracaine was based only on liposomes carrying their content onto the skin, 1.1 $\mu g$ tetracaine should have been found in the skin. Experimental data, however, showed the presence of 369.9 $\mu g$ tetracaine, a 336 times higher amount of drug that would have been expected on the basis of liposome encapsulation. Similarly to liposome formula 1F2, a 317 times enhancement could be found for liposome formula 23C2. These calculations simply meant to illustrate the complexity of liposomal dermal delivery. The amount of tetracaine detected within the skin is greater than the expected amount by direct liposome penetration indicating a complex role for liposomes in promoting cutaneous absorption. It appears that direct intact liposome penetration alone cannot explain the high rate of penetration of liposome encapsulated tetracaine and a "penetration enhancing" effect is also present. The mechanism is being further investigated.

## ACKNOWLEDMENTS

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