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Delivery of Erythromycin to Subcutaneous Tissues in Rats by Means of a Trans-phase Delivery System

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Abstract

Topical administration of antibiotics is associated with reduced risk of systemic side-effects and alteration of gut microflora, and results in higher concentrations of antibiotics at the site of application (and so a lower dose of the drug is required). In conditions such as acne vulgaris, infiltration of the antibiotics into the infected subcutaneous layers is highly desirable. A trans-phase delivery system (TPDS), a mixture of benzyl alcohol, acetone and isopropanol, has been shown to enhance the effective transport of the antibiotic erythromycin across the epidermal barrier and enhance accumulation in the dermis.

Two formulations containing *N*-methyl[^{14}C]erythromycin were compared, a TPDS solution and a propylene glycol solution. They were applied to the dorsal areas of 4–6 week old Fischer rats and tissues were removed for analysis of radioactivity after 2, 4, 8, 12 or 24 h and skin was biopsied and sectioned for autoradiography. The erythromycin dissolved in the TPDS solvent mixture penetrated the stratum corneum and a relatively high concentration was maintained in adjacent tissues for up to 24 h. Penetration was very effective and the erythromycin was detected in significant amounts in the underlying muscle, various organs and later in the urine. In contrast the propylene glycol carrier, probably because of its primarily hydrophilic character, caused the erythromycin to traverse tissue barriers rapidly and appear in the urine. Microautoradiographs qualitatively revealed progressive disappearance of radioactivity from the surface; this correlated with results obtained by direct isotope counting. The route of penetration, in addition to following the interkeratinocyte spaces, seemed to include the perimeter of the pilosebaceous glands and their appendages before diffusion into the capillaries. The propylene glycol solution seemed to traverse the epidermis and the papillary and reticular dermis more rapidly, which might explain its rapid appearance in the urine. These data suggest that the different solutions penetrate the skin by different mechanisms.

Erythromycin macrolide, produced by *Streptomyces erythraeus*, is available as a base, and as the stearate, estolate, ethylsuccinate, gluceptate, lactobionate, or ethylsuccinate (Eady & Cove 1990). It is concentrated in the liver, is excreted and concentrated in the active form in the bile, and is partially deactivated by demethylation in the liver. The serum half-life is approximately 1·4 h (Washington & Wilson 1985).

Transdermal delivery would eliminate first-pass metabolism in the liver and the variable absorption associated with gastrointestinal transit, while improving patient compliance and reducing the incidence of gastrointestinal distress often associated with oral intake (Scheuplein 1976; Patel & Vasavada 1988; Wiegand et al 1992; Sing & Singh 1993). Many investigations of this mode of delivery have used compounds that interact with the stratum corneum barrier of the skin, enhancing the penetration of co-administered compounds (Hori et al 1989). Penetration enhancers such as piperidone and pyrrolidone derivatives (Quan et al 1990; Sasaki et al 1991), oleic acid (Francoeur et al 1990; Takahashi et al 1991), 1-dodecylazacycloheptan-2-one (azone) (Stoughton 1982), cetyl lactate (Kaiho et al 1989), propylene glycol (Nomura et al 1990),

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and transparent oil–water gels (DeVos et al 1991) have been shown to aid the passage of drugs such as the non-steroidal anti-inflammatory compounds across the stratum corneum; benzyl alcohol preparations have also shown promise (Jimbo 1983; Hiramatsu et al 1990). Recent studies in our laboratory compared the levels of 2-[^{14}C]indomethacin in blood, urine, faeces and several tissues after oral and topical delivery to rats (Mikulak et al 1998). The results showed that a trans-phase delivery system containing a mixture of benzyl alcohol, isopropanol and acetone resulted in higher tissue levels of indomethacin in the vicinity of the site of application, and delayed systemic absorption, compared with a standard 50 : 50 (v/v) propylene glycol–ethanol vehicle and an orally administered corn-oil solution.

We have previously shown that benzyl alcohol, as part of an anhydrous solvent system, behaved as a very effective transdermal carrier of griseofulvin in a study comparing oral administration with different topical routes of administration (Nimni 1989; Nimni et al 1990). In this study we have compared benzyl alcohol and propylene glycol, a widely used penetration enhancer for transdermal drug delivery (Priborsky et al 1987; Flynn & Stewart 1988; Sodicoff et al 1990; Celesti et al 1993).

Materials and Methods

Solutions of the active compound

N-Methyl[^{14}C]erythromycin (specific activity 54 mCi mmol^{-1}, radioactive purity 97%; lot number 2469-192) was purchased from New England Nuclear.

Two solutions were prepared containing 1% of the compound. One was in an anhydrous carrier-solvent trans-phase delivery system (TPDS) comprising benzyl alcohol 10%, acetone 40%, and isopropanol 50% (Nimni 1989). Because of its high boiling point benzyl alcohol is considered a stable solvent capable of carrying the erythromycin through the epidermis to the basal layer and across it. The acetone and isopropanol are present as volatile solvents which aid the initial spreading of the preparation and are subsequently mostly eliminated by evaporation. We labelled the system TPDS because, as the volatile solvents dissipate, the solutes become concentrated in the less volatile benzyl alcohol, which then acts as a vehicle for penetration.

The other solution was prepared in propylene glycol, a commonly used penetration enhancer.

Animals and procedures

Fischer 344 rats, 6 weeks old, 150–200 g, were obtained from Simonsen Industries. After topical administration of erythromycin the rats were placed in individual metabolic cages for the duration of the experiment to enable separate collection of faeces and urine. Each value in this study represents triplicate assays from three different rats. Two additional rats per time interval were used for autoradiography. Rats were tranquillized with halothane USP as inhalation anaesthetic. Their right shoulders were shaved so that an area approximately 1 cm square could be drawn on the intact skin over the right glenohumeral joint. Rats were then left to rest for 4 h before application of the drug. The first rat received radioactive erythromycin in TPDS solution (1%, 0·1 mL, 2 μCi), delivered in three separate applications of 33 μL by micropipette. The solution was left to dry and to penetrate the skin for 30 min; any excess was then blotted dry three times with a paper towel. The rat was placed in a metabolic cage and left to recover from the anaesthesia. At different times after the initial application (0·5, 2, 4, 8, 24 and 48 h) rats were killed by intraperitoneal injection of Eutha-6 (1 mL) and the skin was wiped with ethanol to remove any residual surface radioactivity. The rats were dissected and the following samples were taken for assay of radioactivity: skin below the application area, deltoid (a full thickness section 3 mm above and 3 mm below the glenohumeral joint), gluteus maximus, liver, kidney, stool from the ascending colon, and urine from the bladder and from the collection beaker at the base of the metabolic cage. Samples were diced with a scalpel into the smallest possible fragments, weighed, transferred to centrifuge tubes for addition of 2–4 mL acetone, and homogenized in a Polytron homogenizer. The resulting emulsion was centrifuged at 3500 rev min^{-1} for 5 min, and a sample of the erythromycin-containing supernatant was counted for radioactivity (Beckman liquid-scintillation counter). Results are expressed as counts min^{-1} (g tissue)$^{-1}$ or, for urine, per total volume collected. Results are presented as means ± standard deviations (s.d.). During the same experiment another group of rats underwent the same procedure after topical application of radioactive erythromycin in propylene glycol (1%, 0·1 mL, 2 μCi).

Light-microscope autoradiography

A 6-mm diameter skin biopsy was prepared within the marked square. The piece was placed on a glass slide containing a drop of embedding compound and immediately frozen with a cryospray. Full-

thickness tissue slices, 7 μm thick, were cut at -20°C by means of a cryostat. The slices obtained were placed in an autoradiography cassette for 24 h in direct contact with Hyperfilm β-max. The dry slices were then stained with Paragon Blue. This is a variation of the methods of Franz et al (1981) and Sun et al (1994). Adjacent sections were also stained with haematoxylin–eosin.

Results and Discussion

Urinary excretion of N-*methyl[^{14}C]erythromycin*

Use of the TPDS resulted in much less erythromycin being excreted in the urine in the first few hours and much more after 24 h, compared with use of propylene glycol as carrier (Table 1). Consistent with the radiochemical measurement in tissues it would seem that the propylene glycol caused the erythromycin to traverse the dermal barrier more rapidly so that most was excreted within the first 2 h, reflecting the rapid passage across the skin and the relatively smaller amounts of material within the stratum corneum.

Concentration of N-*methyl[^{14}C]erythromycin in internal organs*

Erythromycin is normally metabolized in the liver, where low levels are observed after 2 h (Table 2). When erythromycin was applied in the TPDS a higher steady-state concentration was maintained in the liver from 4 h onwards, reflecting slower release from the dermis. Results from rat kidneys were similar. Similar trends were observed in the underlying muscle at the point of application and the distal gluteus maximus muscle. After 4 h there was a major significant increase in the concentration of erythromycin in the muscle mass just below the site of application, and radioactivity persists at a much higher level than when the erythromycin is applied in propylene glycol.

When, on the other hand, *N*-methyl[^{14}C]erythromycin is dissolved in propylene glycol it seems to reach higher levels much more rapidly and higher concentrations are found in both the liver and kidneys. Perhaps because of the hydrophilic qualities of this penetration enhancer and as a reflection of a different penetration pathway, a higher peak is seen in the kidneys after 4 h, before the more significant peak in the liver at 8 h, coinciding with its rapid excretion in the urine.

Concentration of N-*methyl[^{14}C]erythromycin in the skin—radiochemical analysis and microautoradiography*

The solution containing benzyl alcohol (the TPDS) enables a much larger amount of erythromycin to accumulate in the skin, resulting in a greater continuously diffusing gradient. The drug also persists for longer periods of time (Table 3). When, on the other hand, the *N*-methyl[^{14}C]erythromycin is dissolved in propylene glycol, its concentration

Table 1. Urinary excretion of *N*-methyl[^{14}C]erythromycin at different times after application to the skin surface in different vehicles.

Vehicle	Time (h)	Counts min^{-1} [^{14}C][a]
Trans-phase delivery system	2	1940 ± 1439
Trans-phase delivery system	4	11 750 ± 7541
Trans-phase delivery system	8	4475 ± 4487
Trans-phase delivery system	24	51 096 ± 21 400
Propylene glycol	2	175 600 ± 110 300
Propylene glycol	4	7400 ± 9962
Propylene glycol	8	4020 ± 964
Propylene glycol	24	4320 ± 362

[a]Radioactivity is expressed as counts min^{-1} per total volume of urine collected.

Table 2. Distribution of *N*-methyl[^{14}C]erythromycin in the internal organs of rats at different times after application to the skin surface in different vehicles.

Vehicle	Time (h)	Counts min^{-1} [^{14}C] accumulated[a]	
		Kidney	Liver
Trans-phase delivery system	2	79 ± 24	46 ± 11
Trans-phase delivery system	4	387 ± 203	271 ± 68
Trans-phase delivery system	8	234 ± 135	246 ± 65
Trans-phase delivery system	24	327 ± 184	335 ± 129
Propylene glycol	2	279 ± 35	506 ± 88
Propylene glycol	4	690 ± 172	401 ± 17
Propylene glycol	8	206 ± 5	2300 ± 682
Propylene glycol	24	32 ± 11	17 ± 2

[a]Radioactivity is expressed as counts min^{-1} (g wet tissue)$^{-1}$.

Table 3. Skin levels of *N*-methyl[^{14}C]erythromycin in rats, at the site of application, at different times after application in different vehicles.

Vehicle	Time (h)	Counts min^{-1} [^{14}C] accumulated
Trans-phase delivery system	2	393 968 ± 193 300
Trans-phase delivery system	4	510 033 ± 108 420
Trans-phase delivery system	8	645 457 ± 148 370
Trans-phase delivery system	24	219 394 ± 24 340
Propylene glycol	2	193 670 ± 77 748
Propylene glycol	4	47 661 ± 43 300
Propylene glycol	8	77 986 ± 49 590
Propylene glycol	24	10 006 ± 3028

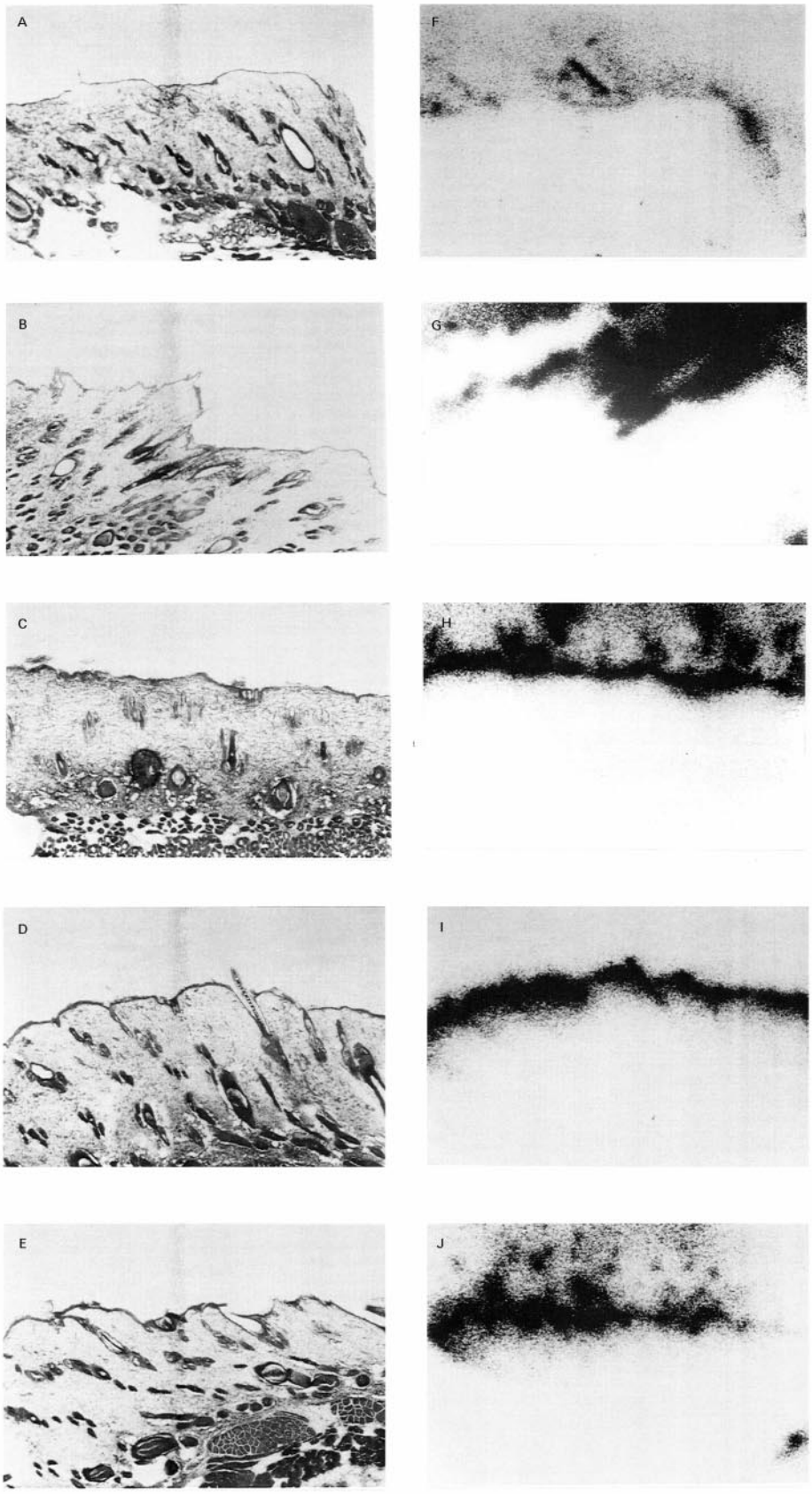

Figure 1. Microautoradiographs of frozen skin specimens obtained at different times (A and F, 0 h; B and G, 2 h; C and H, 4 h; D and I, 8 h; E and J, 12 h) after the application of 1 μCi *N*-methyl[^{14}C]erythromycin to the skin surface in the trans-phase delivery solvent system (magnification 10×). A–E. haematoxylin–eosin staining; F–J. autoradiography.

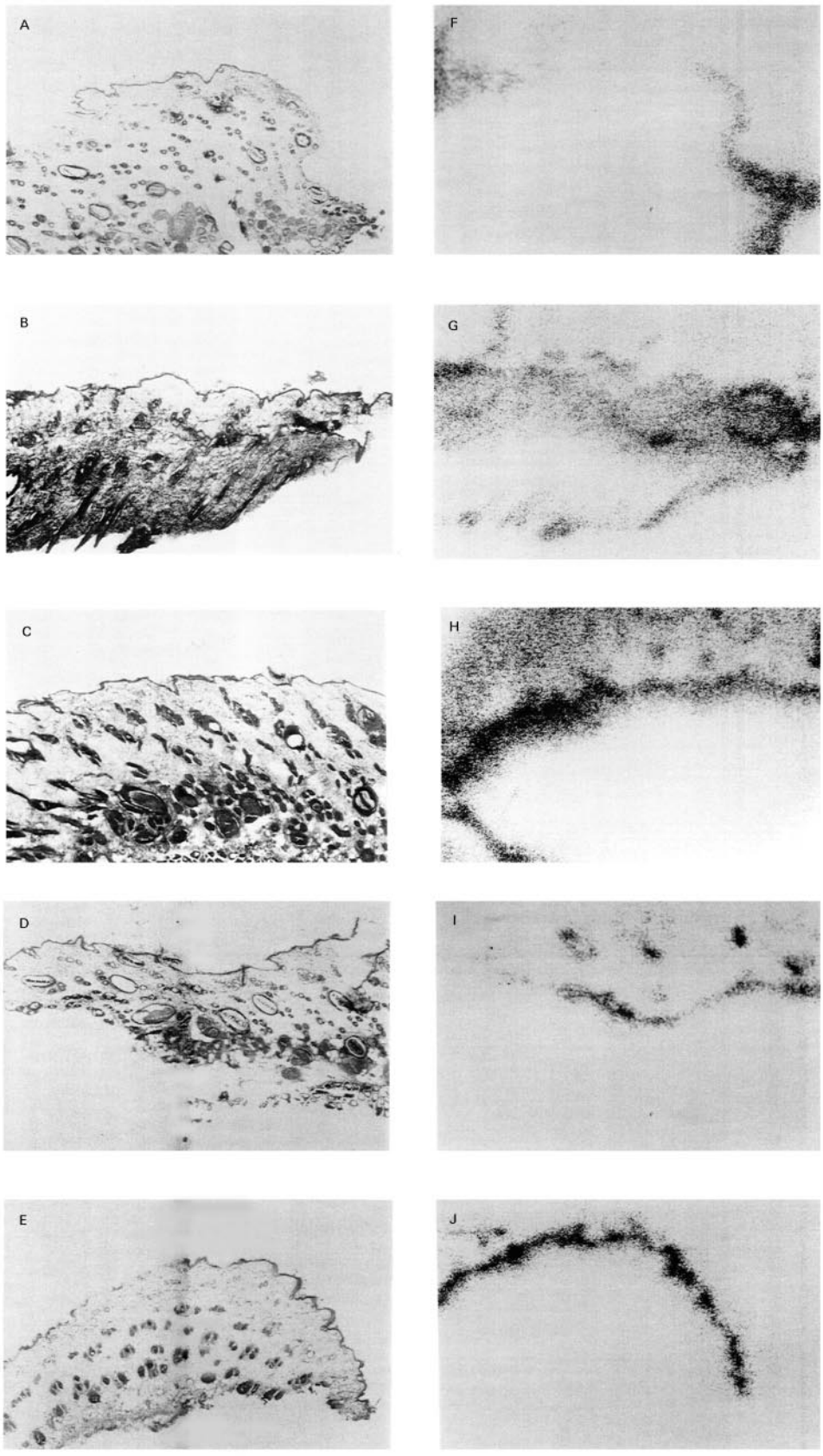

Figure 2. Microautoradiographs of frozen skin specimens obtained at different times (A and F, 0 h; B and G, 2 h; C and H, 4 h; D and I, 8 h; E and J, 12 h) after the application of 1 μCi *N*-methyl[^{14}C]erythromycin to the skin surface in propylene glycol (magnification 10×). A–E. haematoxylin–eosin staining; F–J. autoradiography.

peaks initially but is followed by a rapid decline consistent with low retention. Results from microautoradiography are indicative of slow diffusion through the epidermal and dermal layers (Figure 1). At time zero, after removal of the excess surface material, the applied drug remains essentially coating the outer epithelial layer and 2–4 h later is seen rapidly diffusing through the stratum corneum. When dissolved in the TPDS the radioactive compound persists in the epidermis longer. The distribution of darker staining material above the epidermis reflects an artefact associated with processing—it results from migration of radioactivity from the surface of the epidermis into the embedding media during the freezing process. Nevertheless the intensity of the stain represents the total amount of radioactivity included in the skin at the time the specimen was explanted for freezing (after surface wiping). The initial barrier to be traversed is the stratum corneum; after this there is slow diffusion across the epidermis and the papillary dermis, the dermal layer closer to the epidermis. Eight hours after application with TPDS there is a tendency of the drug to partition into the appendages of the skin, including the hair shafts and the pilosebaceous glands. The vasculature serving the hair follicle could serve as a point of access for entry of the drug into the systemic circulation.

The initial concentration was much less for drug dissolved in propylene glycol and the concentration decreased rapidly and continuously with time. After 2 h the microautoradiograph qualitatively shows rapid diffusion across the skin layer; diminishing amounts of radioactivity remain after longer periods (Figure 2). This is consistent with the radiochemically determined concentrations of erythromycin (Table 3)—a large concentration of erythromycin in the skin after 2 h decreases markedly with time.

The solubility characteristics of the erythromycin base used in this study might also contribute to the different behaviour associated with the two solvent systems. Whereas erythromycin is only slightly soluble in water (0·1%) it is freely soluble in organic solvents such as alcohol, chloroform, ether, acetone, acetonitrile and benzyl alcohol, which might enable its transfer to the lipoidal components of cells and matrices. The results from microautoradiography seem similar to those of Franz et al (1981) who observed progressive disappearance of radioactivity from the surface and a route of penetration which initially seemed to occur adjacent to the pilosebaceous glands and skin appendages, followed by diffusion of the labelled molecules through the epidermis before subsequent capillary transport. The erythromycin dissolved in the TPDS might penetrate the skin by various routes. One might include the follicular pores, where the hair shafts exit, and which are in communication with the pilosebaceous glands; another might be through the lipid-loaded interstices between the epidermal cells. In general, the duct of the gland is filled with a soft, slowly extruded lipoidal medium, sebum, and drug dissolved in the lypophilic solvent mixture tends to accumulate in this area. This might be followed by diffusion through the sebum into the surrounding dermis, therefore, the pilosebaceous glands providing an accessory, although quantitatively less important, mechanism for penetration (Franz et al 1981). When the TPDS is used as carrier, diffusion might involve migration from sebum to epidermis and then steady entry into the systemic circulation. This might contribute to the threefold greater concentration of erythromycin in the skin and for its persistence when administered in the lypophilic vehicle. Propylene glycol, on the other hand, carries the drug through the skin rapidly, possibly by a different pathway; this might explain its faster excretion and disappearance from the site of application. Although the exact nature of these apparently distinct pathways has not yet been elucidated, these data provide additional evidence for their existence and should encourage others to pursue this important area of research.

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