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Percutaneous Absorption of Salicylates from Some Commercially Available Topical Products Containing Methyl Salicylate or Salicylate Salts in Rats

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Abstract

Studies to determine the extent of local tissue penetration of topically applied, commercially available salicylate esters and salts were conducted in male Wistar rats.

The salicylate concentration in plasma, tissues underlying the site of drug application, and similar tissues on the contralateral (control) side were measured. The plasma and tissue salicylate levels suggest that direct penetration of salicylate was predominant to the top muscle level on the treated site. Results also suggest that the drugs were first absorbed into the bloodstream and subsequently distributed to both the deeper tissues on the treated site and the contralateral tissues. The topical application of formulations of ester methyl salicylate and salts triethanolamine salicylate and diethylamine salicylate containing comparable salicylate concentrations yielded similar salicylate concentrations in the various tissues.

The salicylate concentrations in the deeper tissues approached concentrations observed in the contralateral tissues suggesting that salicylate present in these tissues was due to the systemic blood supply.

In recent years, there has been a significant increase in the number of topical formulations that require deep-tissue penetration to produce their desired localized therapeutic effect. Salicylates are common active ingredients in most topical analgesic creams used for joint stiffness, strains and sore muscles. The creams are also used as adjuncts for arthritis and rheumatism therapy (Roberts et al 1982; Babar et al 1990). The percutaneous absorption of methyl salicylate in man (Roberts et al 1982), and of salicylate esters and other non-steroidal anti-inflammatory drugs (NSAIDs) in man (Yano et al 1986) has been documented. The metabolism of salicylate esters in the body after topical application has been studied by Danon et al (1986) and Guzek et al (1989). The latter group used human skin grafted to athymic mice to demonstrate enzymatic cleavage of the diester to the monoester and salicylic acid. Direct penetration and localization of salicylates in tissues underlying the site of drug application has been demonstrated in pigs (Baldwin et al 1984) and dogs (Rabinowitz et al 1982; Rabinowitz & Baker 1984). Singh & Roberts (1993a) reported that direct tissue penetration of salicylic acid after dermal application in rats is dominant only to a depth of 3–4 mm. Limited information is available on the depth of penetration of the ester and salts of salicylates following topical application of commercially available products. This study was undertaken to determine the depth of penetration of salicylates following topical application of commercially available products, and to compare the penetration of salicylate from commercial preparations containing salicylate esters and salicylate salts.

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Materials and Methods

Formulations

Commercially marketed creams were investigated in this study. The products evaluated were Dencorub cream (Carter Wallace Aust. Pty, Ltd, NSW: Batch No. 4BD51), Metsal cream (3M Pharmaceuticals Pty, Ltd, NSW: Batch No. 9062C), Biosal cream (Yauyip Pty, Ltd, NSW: Batch No. 60647), Deep Heat cream (Mentholatum Pty, Ltd, Victoria: Batch No. A472), and Goanna rub (Herron Pharmaceuticals Pty, Ltd, Queensland: Batch No. 7257). These contain 20, 28·3, 12, 12·74 and 10% methyl salicylate, respectively. Dencorub cream (Carter Wallace Aust. Pty, Ltd, NSW: Batch No. 2C1) and Goanna Arthritis cream (Herron Pharmaceuticals Pty, Ltd, Queensland: Batch No. 9052) each contained 10% triethanolamine salicylate. Rubesal cream (Hamilton Laboratories, Victoria: Batch No. 31) containing 10% diethylamine salicylate was also evaluated.

Chemicals

Salicylic acid, methyl salicylate and *p*-toluic acid were purchased from Sigma Chemical Company, USA. HPLC grade acetonitrile was from Rhône Poulenc Chemical Pty, Ltd, Australia. Phosphoric acid, methanol and perchloric acid used were of analytical grade.

Absorption studies

Each preparation (salicylic acid equivalent of 18·9 and 10 mg cm^{-2} for the ester and salts, respectively) was applied onto a 9·625 cm^2 depilated (Nair cream) abdominal skin of anaesthetized (phenobarbitone sodium, 60 mg kg^{-1}, i.p.) male Wistar rats (308·0 ± 17·0 g). Dosing uniformity between rats was ensured by marking the actual skin area with a template and indelible ink. The formulation was removed from the rat skin with a spatula 2 h post-application. A blood

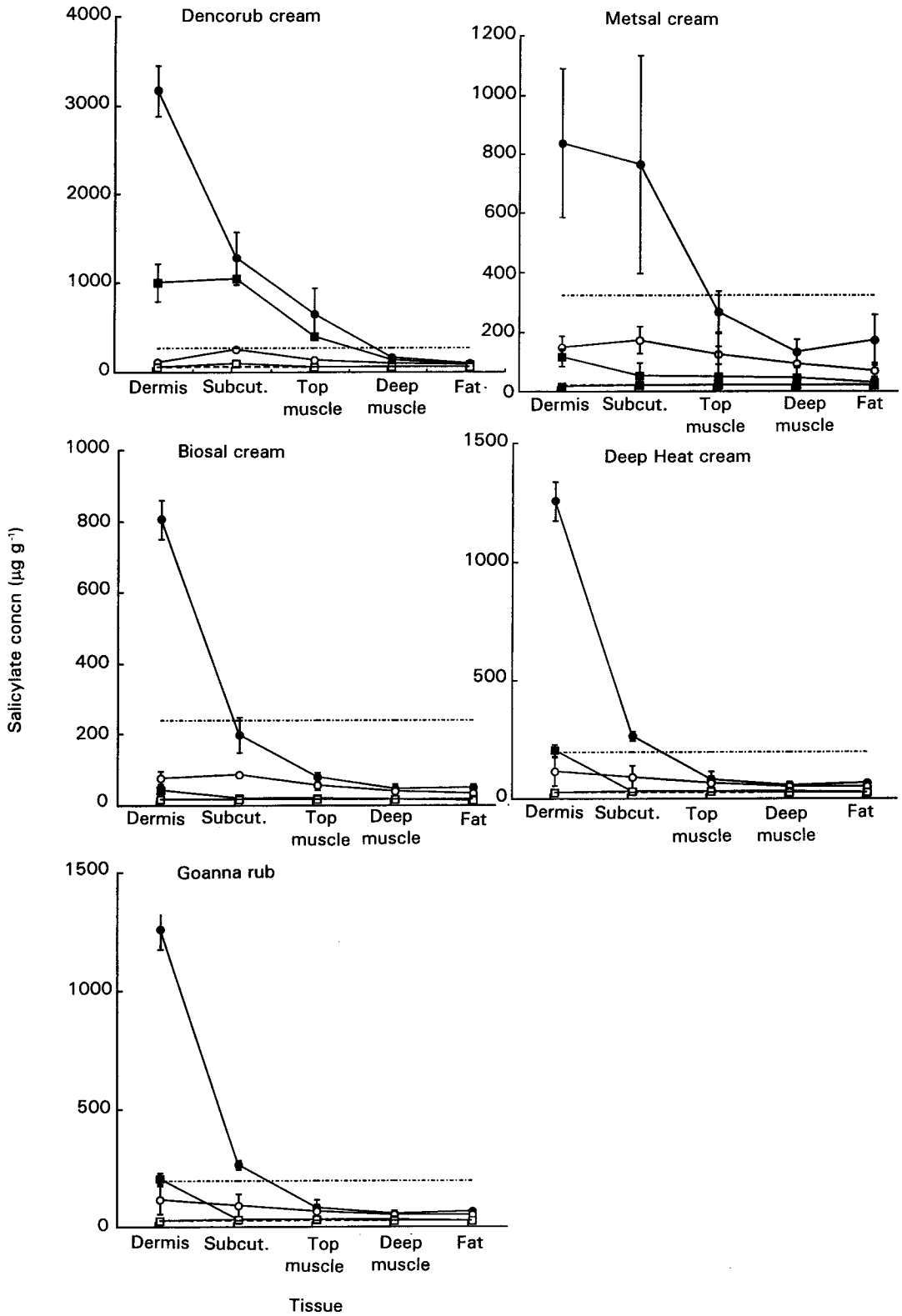

FIG. 1. Tissue concentration (mean ± s.e.m., n = 3) of salicylate following topical application of the ester, methyl salicylate formulations Dencorub cream, Metsal cream, Biosal cream, Deep Heat cream and Goanna rub. ■ Treated tissue, □ contralateral tissue, and ---- plasma for methyl salicylate; ● treated tissue, ○ contralateral tissue, and -·-·- plasma for salicylic acid.

sample was then taken from the tail vein, before the animal was killed by cervical dislocation. The blood sample was centrifuged. Tissue samples (skin, subcutaneous, top muscle, deep muscle and fat) below the site of drug application were immediately and sequentially excised. Contamination between different tissue layers was prevented by thorough wiping of the dissecting scissors and forceps with methanol after each tissue separation. The epidermis was separated from the dermis by exposure of the excised skin to ammonia fumes for 1 h followed by removal with a surgical blade. The epidermis was discarded. Tissues were similarly excised from the contralateral side. Tissue and plasma samples were stored at −20°C before analysis for salicylate. The experimental procedure was carried out in triplicate for each of the preparations.

Sample treatment

Plasma (100 μL) was pipetted into a microfuge tube containing the internal standard, 2 μg mL^{-1} *p*-toluic acid in acetonitrile (200 μL) and 35% perchloric acid (20 μL). The sample was thoroughly vortexed. The tube was centrifuged and the clear supernatant removed. Each tissue type was minced with scissors and 100 mg transferred into a microfuge tube. Acetonitrile (400 μL) containing 2 μg mL^{-1} *p*-toluic acid as internal standard and 35% perchloric acid (20 μL) were added (Rumble et al 1981; Owen et al 1987). The sample was thoroughly vortexed and ultrasonicated on ice for 30 s. The tube was centrifuged and the supernatant removed.

Assay

Salicylate concentration in the collected supernatant was analysed by high-performance liquid chromatography using a modification of the assay described by Rumble et al (1981). The analysis was carried out using a 300 mm length, 10 μm C_{18} μ Bondapak column (Waters) with a 3·9 mm internal diameter, 7 μm precolumn (RP-18, Brownlee Newguard) and a 237 nm UV detector (Perkin Elmer LC 90). The mobile phase consisted of acetonitrile, 0·03% phosphoric acid, triethylamine (30:70:1), pH 2·5 and was pumped through the column at 2 mL min^{-1} using an isocratic pump (Perkin Elmer LC 250). Fifty microlitres of supernatant was injected automatically (Shimadzu SIL 9A). Salicylic acid, *p*-toluic acid and methyl salicylate eluted at 4·92, 6·06 and 15·20 min, respectively, under these conditions. The detection limit was 5 ng per 50 μL injected.

Statistics

A two-way analysis of variance was used and the level of significance was $P < 0{\cdot}05$. At least three rats were used for each formulation.

Results

Fig. 1 shows the typical tissue concentration-depth distribution profiles of methyl salicylate after 2 h of epidermal application of the ester. Also shown in Fig. 1 is the salicylic acid present in each tissue after topical administration of methyl salicylate. It is apparent that methyl salicylate is mainly converted to its major metabolite, salicylic acid, during transport through the skin. Salicylic acid metabolites

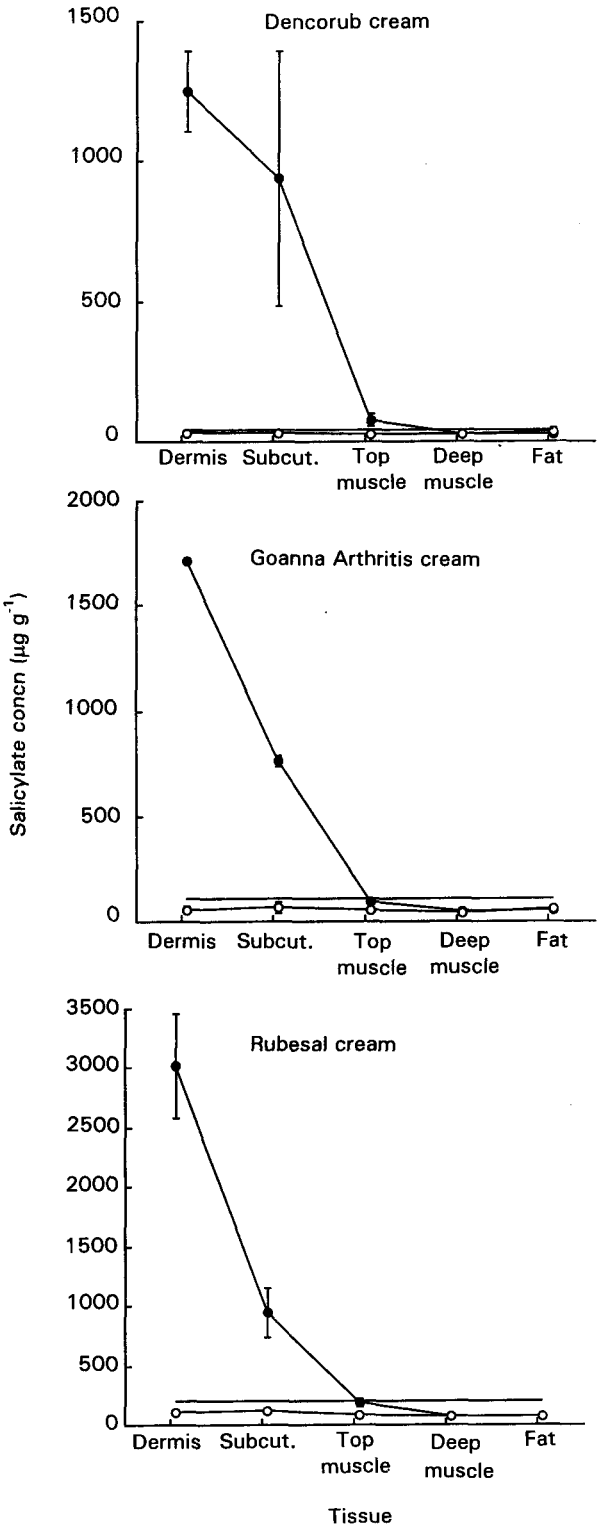

FIG. 2. Tissue concentration (mean ± s.e., n = 3) of salicylate following topical application of the salts, triethanolamine salicylate and diethylamine salicylate formulations Dencorub cream, Goanna Arthritis cream, and Rubesal cream. ● Treated tissue, ○ contralateral tissue, and —— plasma.

Table 1. Salicylate recovery from various tissues expressed as treated to contralateral ratios after epidermal application of the various commercial products for 2 h.

Products	Dermis	Subcutaneous	Top muscle	Deep muscle	Fat
Dencorub cream	22·3 ± 4·59	12·7 ± 2·50	3·0 ± 0·53	1·2 ± 0·26	—
Metsal cream	6·8 ± 1·47	5·2 ± 1·73	3·1 ± 0·82	1·8 ± 0·47	6·0 ± 4·43
Biosal cream	9·5 ± 3·61	2·1 ± 0·22	1·3 ± 0·36	1·2 ± 0·45	2·7 ± 1·09
Deep Heat cream	11·7 ± 0·95	2·9 ± 0·69	1·4 ± 0·10	1·2 ± 0·08	1·3 ± 0·51
Goanna rub	13·5 ± 0·97	3·1 ± 0·29	1·3 ± 0·10	1·1 ± 0·08	2·3 ± 1·49
Rubesal cream	142·0 ± 4·53	73·7 ± 3·94	12·8 ± 1·23	1·9 ± 0·64	0·5 ± 0·23
Dencorub cream[a]	55·4 ± 4·53	35·0 ± 3·94	2·8 ± 1·23	1·7 ± 0·64	0·9 ± 0·23
Goanna Arthritis cream[a]	63·6 ± 8·29	16·8 ± 3·31	6·8 ± 2·44	1·2 ± 0·21	1·8 ± 0·76

[a] Containing 1% triethanolamine. Values are mean ± s.e.m.

were not detected in either the tissues or plasma under the assay conditions used in this study. The tissue distribution of methyl salicylate from Dencorub cream and Goanna rub are higher in the dermis, subcutaneous tissue and top muscle underlying the treated site after application of all formulations relative to plasma concentrations and concentrations in similar tissues on the contralateral side. Fig. 2 shows that salicylic acid administered as its salts has higher concentrations in the dermis, subcutaneous tissue and the top muscle below the treated site than in plasma and contralateral tissues. The concentrations in deeper underlying tissues (deep muscle and fat) are lower than the plasma concentration but comparable with the observed contralateral tissue concentrations.

Table 1 shows the treated tissue:contralateral tissue concentration ratios of salicylate in various tissues for the different formulations studied. Although the mean ratios in the dermis, subcutaneous tissue and top muscle for methyl salicylate are lower than either triethanolamine salicylate or diethylamine salicylate, there is considerable variability in the data obtained. Fig. 3 shows the ratio of methyl salicylate to total salicylate for varying tissue depths.

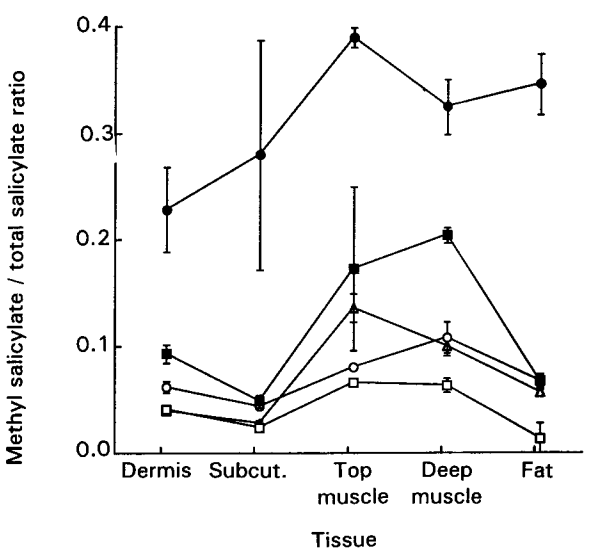

FIG. 3. Methyl salicylate to total salicylate concentration ratios (mean ± s.e.m., n = 3) on the treated site following topical application of Dencorub cream (●), Metsal cream (△), Biosal cream (○), Deep Heat cream (□) and Goanna rub (■).

The extent of hydrolysis of methyl salicylate was generally greater in the dermis and subcutaneous tissue as compared with the deeper tissues. Table 2 shows salicylate concentrations in plasma and various tissues after application of products containing a comparable amount of salicylate (10%). Similar tissue concentrations were observed at varying depths below the treated site despite the variabilities recorded.

Discussion

The results in Fig. 1 suggest that direct penetration of salicylate from methyl salicylate formulation occurs throughout the tissues below the treated site, as concentrations in all tissues are greater than in contralateral tissues. In contrast, the salicylate levels are identical for the salts in deep muscle and fat in both the treated and contralateral sites. The higher levels from methyl salicylate probably reflect the diffusion of the lipophilic methyl salicylate into deeper tissues and its poorer metabolism to salicylic acid with increasing tissue depth.

This conclusion is supported by results presented in Figs 2 and 3. Appreciable amounts of methyl salicylate were detected in all the tissues (Fig. 2). The high methyl salicylate:total salicylate ratios obtained with the deeper tissues is an indication of poor metabolism of the substrate in these tissues. This result is in accordance with histochemical observations in man where unspecific esterases were found to be distributed mainly within the epidermal layer (Braun-Falco 1963; Wohlrab et al 1968). The detection of methyl salicylate mainly as salicylic acid observed in this study is consistent with the reported metabolism of methyl salicylate to salicylic acid (Behrendt & Kampffmeyer 1989). Behrendt & Kampffmeyer (1989) studied the absorption and cleavage of methyl salicylate by skin of single-pass perfused ears of the rabbit. They reported that methyl salicylate was hydrolysed to salicylic acid with an apparent V_{max} of 1·5 nmol min^{-1} cm^{-2}, a rate about 25 times greater than after arterial administration. Guzek et al (1989) reported the hydrolysis of a salicylate diester to methyl salicylate and salicylic acid. Those authors noted an exponential decrease in salicylate ester concentration with increasing depth (up to about 300 μm) after topical application of the diester to human skin grafted to athymic mice. Guzek et al (1989) concluded that the hydrolysis of the di- and monoester to salicylic acid is due to skin esterase activity. Wiegrebe et al

Table 2. Comparison of salicylate concentrations in tissues and plasma following the application of the ester and the salt formulations containing equivalent salicylate concentrations.

Product/drug	Salicylate concentration[a]					
	Dermis[b]	Subcutaneous	Top muscle	Deep muscle	Fat	Plasma[c]
Goanna rub	1417 ± 60	254 ± 12	70 ± 2	43 ± 1	45 ± 7	175 ± 25
Rubesal cream	1229 ± 8	920 ± 26	55 ± 1	5 ± 0	5 ± 0	22 ± 3
Dencorub cream	1686 ± 65	734 ± 36	64 ± 6	17 ± 1	29 ± 5	80 ± 18
Goanna Arthritis cream	2970 ± 44	899 ± 20	142 ± 3	28 ± 6	21 ± 3	114 ± 39

[a] Mean ± s.e.m., [b] $\mu g\,g^{-1}$, [c] $\mu g\,mL^{-1}$.

(1984) had previously reported that hydrocortisone 17-butyrate 21-propionate is hydrolysed in dog skin to the monoester and hydrocortisone. Table 1 shows ratios are highest in the most superficial layers of the skin. A ratio approaching unity is to be expected when blood perfusion dominates the transport of salicylic acid into the various tissues. Ratios much greater than unity are suggestive of direct penetration. The high ratio obtained for all the products suggests that salicylic acid distribution down to the subcutaneous tissue is mainly by direct penetration. A two-way analysis of variance of data in Table 1 indicates that for the salts, penetration also occurs down to the top muscles. The inter-subject variations in all cases were not significant. There was no significant difference in the ratios for Dencorub cream and Goanna rub in tissues below the top muscle and these values approach unity. It is suggested that salicylate delivery to these tissues is mainly occurring via the systemic circulation.

Singh & Roberts (1993a) also reported tissue uptake and localization of salicylates and other NSAIDs following dermal application. They compared the depth of penetration of various NSAIDs and found an equivalent depth of penetration for this group of compounds. Using a tissue diffusion pharmacokinetic model, Singh & Roberts (1993b) modelled salicylate penetration into deeper tissues and derived an intertissue diffusion coefficient of an order comparable with known tissue diffusion coefficients. In the present work, salicylate was applied topically in contrast to Singh & Roberts' (1993a, b) dermal application studies. We obtained a depth of penetration of 3–4 mm due to direct penetration. This value confirms the result obtained by Singh & Roberts (1993a, b). McNeill et al (1992) studied the penetration of piroxicam, another NSAID, in rats. The marked difference in the concentration-time profiles for the treated and non-treated sites found cannot be solely attributed to a redistribution of the piroxicam into deeper tissue by the cutaneous blood supply. McNeill et al (1992) suggested that the cutaneous microvasculature is not an infinite sink for removal of all topically applied drugs. Monteiro Riviere et al (1993) also hypothesized that the local vasculature underlying the application site creates a convective force carrying topically applied drug down into the underlying tissues without being absorbed into the systemic circulation. A two-way analysis of variance of the data presented in Fig. 3 indicates that the inter-subject variations observed were not significant. There was also no significant difference in the methyl salicylate:total salicylate ratios obtained for Dencorub cream and Goanna rub in tissues below the top muscle. This confirms that salicylate delivery to these tissues is mainly due to a redistribution of the drug by the cutaneous blood supply.

This study suggests that salicylate does penetrate to tissues to the level of the muscle below the area of drug application. The metabolism of methyl salicylate occurs more within the skin than in the deeper tissues (muscles). The salicylate ester penetrated better than the salts.

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