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Effect of Ion Pairing with Alkylamines on the In-vitro Dermal Penetration and Local Tissue Disposition of Salicylates

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Abstract

Hydrophilic ionic drugs can be rendered lipophilic by ion-pair formation with hydrophobic counter-ions. This study examines the value of forming ion pairs between anionic salicylate and a series of amines as model cationic counter-ions to facilitate topical delivery and skin penetration. The in-vitro translocation of salicylate ions from a non-aqueous vehicle through human epidermis was estimated in the presence or absence of amines. The distribution into, and accumulation of the salicylate ion in various tissues following topical application to anaesthetised rats were also investigated.

Although the epidermal permeation constants of the salicylate-amine ion pairs were lower than that of salicylate itself (enhancement ratios: 0·74–0·87), salicylate retention and localisation in the underlying rat tissues increased in the presence of some of the counter-ions studied. Salicylate concentrations (μg (g tissue)$^{-1}$) in the dermis were $877{\cdot}2 \pm 78{\cdot}6$ for salicylate alone and $1098 \pm 121{\cdot}9$–$2586 \pm 332{\cdot}5$ for salicylate-amine ion pairs. The levels of salicylate in tissues up to the top muscle layer were 1·2–3·7-fold higher in the presence of the counter-ions.

It is concluded that, although amine counter-ions have the ability to influence the penetration of salicylate, in-vitro permeability studies do not reflect the in-vivo increases in tissue concentrations resulting from probable changes in systemic clearance.

Many drugs which may be used for either local or systemic effect following transdermal delivery are weak acids or bases and are thus ionised under normal physiological conditions. Ionised molecules are generally not well absorbed by biological membranes. Human skin is lipoidal in nature and does not allow easy transport of inorganic ions and highly polar substances (Tregear 1966). Optimisation of a topical formulation generally implies that the flux of drug into the skin be maximised. This requirement means that the product of drug concentration in the vehicle and drug partition coefficient between stratum corneum and vehicle be as large as possible (Surber et al 1990).

One possible means of facilitating the transdermal delivery of ionic drugs is through ion-pair formation. Oppositely charged ions can interact to form new species known as ion pairs. This association reduces or neutralises the electrostatic charges and consequently reduces the electrical conductivity in a non-polar milieu. Attempts have been made to utilise ion-pair formation to improve drug absorption. The use of pH gradients to facilitate transport of inorganic ions from an aqueous compartment across a non-polar organic phase into a receptor aqueous phase against the ion concentration gradient has been described (Lee et al 1978; Babcock et al 1980; Thelander et al 1980). This strategy was developed in an attempt to facilitate transport of the anionic moiety across the stratum corneum. In another model experiment using salicylate (anionic drug) and Azone (cationic counter-ion) ion-pair transfer across a model lipophilic membrane was achieved as a result of a pH gradient (Hadgraft et al 1985). Green et al (1988) showed that the increase in the in-vitro flux of naphazoline across human cadaver skin pretreated with fatty acids was due to establishing a pH gradient. The epidermal surface is slightly acidic,

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pH 4·2–5·6 (Katz 1973). The intradermal or physiological pH is 7·3–7·4, so this in-vivo pH gradient across the epidermis may be utilised to provide the free energy for facilitated transfer of an anionic drug. Barker & Hadgraft (1981) showed that a series of N-substituted di-isopropanolamines facilitated the transfer of anionic methyl orange across an isopropyl myristate-supported membrane, against its own concentration gradient, by utilising a pH gradient of 5·0–7·4 as the driving force.

Although the relevance of ion-pair formation to the membrane uptake of ionised molecules has been often discussed, there are very few systematic studies (Ruifrok & Meijer 1981) to examine how and why ion pairs could be employed in this role. A variety of amines have been reported as topical formulatory adjuvants (Poulsen et al 1968; Quack 1976). Reasons for their inclusion have not been adequately investigated. This study was designed to elucidate the possibility of forming ion pairs between a model anionic drug poorly absorbable by the skin, namely salicylate and a series of alkylamines used here as model cationic counter-ions. The effect of these amines on the in-vitro percutaneous penetration of salicylate through human epidermis and the transport and distribution of salicylate ion pairs in anaesthetised male Wistar rats were investigated.

Materials and Methods

Materials

Salicylic acid, sodium salicylate, methylamine, ethylamine, propylamine, butylamine, pentylamine, hexylamine, heptylamine, octylamine, nonylamine, decylamine, undecylamine, dodecylamine, diethylamine, triethylamine, triethanolamine, 2-dimethylamino ethanol (deanol), benzydamine hydrochloride, propylene glycol and isopropyl myristate were purchased from Sigma Chemical Co. (Sydney, Australia). Methanol (HPLC grade) and toluene were obtained from BDH chemicals (Kilsyth, Victoria, Australia). ^{14}C-salicylic acid was purchased from New England Nuclear, USA. All other reagents were of analytical grade and were used as received. A liquid scintillation counter (Tri-carb 4000 series, United Technologies Packard, USA) was used to determine radioactivity in the samples. Ultraviolet spectra were obtained using a UNICON 810/820 spectrophotometer.

Solubility determinations

The solubility of salicylic acid in various vehicles was determined. The vehicles were citric acid-phosphate buffer (pH 5), toluene, isopropyl myristate, light liquid paraffin, or 0·1 M n-alkylamine in each of these four vehicles. Salicylic acid was added to the vehicles in excess of its solubility. The solubility of salicylic acid in distilled water containing 0·0–2·5 M amine adjuvants was also determined. The suspensions were agitated by end-to-end mixing at room temperature (approx. 25°C) for at least 16 h. Preliminary experiments had shown that dissolution equilibrium was attained in this period. Salicylate content in the vehicles was determined after filtration and dilution. Experiments were performed in triplicate and analysis of variance carried out to identify significant changes in
solubility.

Partitioning experiments

Toluene, light liquid paraffin, octanol and isopropyl myristate were used as liquid surrogates for skin lipids. Propylene glycol or phosphate buffer (pH 5) were used as the aqueous phase. The oily and aqueous phases had been pre-saturated with one another by equilibration overnight before these experiments. The samples were dissolved in 10 mL of the aqueous phase. A trace amount of ^{14}C-salicylic acid was added to the salicylic acid solutions. Equimolar concentrations of salicylic acid and the respective amine were used in this study. The distribution coefficients were determined by equilibrating 2 mL of both phases by end-to-end mixing at room temperature ($\approx$25°C) for 16 h. The phases were then separated and salicylate concentrations in both phases were determined by scintillation counting.

To establish whether the amines are capable of forming ion pairs with the salicylate anion, the pH extraction profiles of salicylic acid between buffer solutions and either toluene alone or 0·5 M amine adjuvants dissolved in toluene were determined. Citric acid–phosphate buffers (pH range: 2·5–7·5) were used as the aqueous phase. The procedure was similar to that reported for partition coefficient determination.

Conductivity measurements

The specific conductance of drug solutions (salicylic acid or equimolar concentrations of salicylic acid and the amines in ethanol–propylene glycol (2 : 1 v/v) vehicle) used in permeation studies was measured at room temperature with a conductivity meter (Radiometer, Copenhagen, Model CDM80). Specific conductance, k, was measured by direct reading of the conductivity meter and given by:

$$k = (d/a)G \quad (1)$$

where d and a denote the distance between the electrodes and the area of the electrode respectively; d/a is thus the cell constant and G denotes conductivity in reciprocal ohms. The unit of specific conductance is siemens per cm ($S\,cm^{-1}$).

Preparation of isolated human epidermis

Human skin was obtained from the abdominal region of female patients undergoing cosmetic surgery. The subcutaneous fat was carefully trimmed off and the full-thickness skin washed with deionised distilled water. The epidermis was separated from the dermis by the heat method (Kligman & Christophers 1963). The full-thickness skin freed of subcutaneous fat was immersed in deionised distilled water at 60°C for 3 min. The epidermis was gently peeled off with the thumb. The isolated epidermis was dried between filter papers and kept frozen until required.

Permeation experiments

In-vitro permeation studies across isolated human epidermis were carried out in pyrex glass Franz-type diffusion cells. The membrane was immersed in deionised distilled water for one hour before use. A thin film of lubricant was spread on the lapped glass surfaces of the half cells to ensure water-tight glass-to-membrane seals. Isolated human epidermis, supported on gauze, was mounted between the diffusion cells and the assembly held in place with a plastic clamp. The diffusion unit was then immersed in a water bath at 37°C. Penetration occurred through a cross-sectional area of 1·13 cm^2. The receptor cell has a capacity of about 3·5 mL. Phosphate buffer, pH 7·4 containing 25% ethanol was the receptor fluid. The donor phase was a solution of salicylic acid or equimolar concentrations of salicylic acid–amine counter-ion in ethanol/propylene glycol (2 : 1 v/v). Trace amounts of ^{14}C-salicylic acid were added to salicylic acid. After equilibration with the buffer, 1 mL of the donor solution was added to the donor cell while buffer was introduced into the receptor cell. The receptor compartment was continually stirred by a magnetic stirrer driven by an external magnet. At sampling times, the receptor-cell content was withdrawn and replaced with drug-free buffer maintained at 37°C. The concentration of salicylate in the receptor solution was analysed by liquid scintillation counting. The permeation data represent 3–6 determinations.

Animals

Male Wistar rats (300–350 g) were used in this study. The rats were housed under standard laboratory conditions (20·0 ± 0·5°C, 55–75% r. h.) and allowed free access to normal pellet diet and water. All experiments had previously been approved by the Animal Ethics Experimentation Committees of the University of Queensland and Princess Alexandra Hospital.

In-vivo epidermal penetration and local-tissue uptake studies

The rats were lightly anaesthetised (phenobarbitone sodium, 60 $mg\,kg^{-1}$, i.p.) and their body temperature maintained at 37°C by placing them on a heating pad. The hair from 4 cm^2 abdominal site was depilated (Nair cream). A glass cell of internal diameter 1·8 cm was adhered to the exposed epidermis and warmed to 37°C by means of an external heating device. A solution of salicylic acid or equimolar concentrations of salicylic acid–amine counter-ion in ethanol–propylene glycol (2 : 1 v/v), previously warmed to 37°C, was introduced into the epidermal glass cell and the solution stirred by a glass stirrer driven by an external motor. An equimolar mixture of sodium salicylate and benzydamine hydrochloride in ethanol–propylene glycol (2 : 1 v/v) was also used as the donor phase. A blood sample was taken from the tail vein of the rat at predetermined times, centrifuged and the plasma sample collected. After 6 h, the rat was killed, while still under anaesthesia, by cervical dislocation. Tissue samples (skin, subcutaneous tissue, 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 thoroughly wiping 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 drug concentration by HPLC. The experimental procedure was carried out in triplicate for each preparation.

Sample treatment and analysis

Samples collected during and at the end of the studies were assayed for salicylic acid by either UV spectrophotometry, scintillation counting or by HPLC.

The amount of salicylic acid in the vehicles after solubility experiments was determined by UV spectrophotometry. Salicylic acid showed an absorption maxima at a wavelength of 304 nm in the respective vehicles used for solubility experiments. The samples collected from this set of experiments were adequately diluted and their absorbance measured at 304 nm. The concentration of salicylic acid in the vehicles was obtained by making reference to absorbance vs concentration plots for salicylic acid.

Salicylate concentrations in the aqueous and lipid phases collected after partition experiments and in the receptor phase obtained during in-vitro epidermal permeation studies were determined by liquid scintillation counting. A 100-μL portion of each sample was mixed with 5 mL of liquid scintillation fluid (Ultima Gold, Packard, USA) and counted for radioactivity for 2 min on the liquid scintillation counter. The partition coefficient (P) data represent an average of three determinations and are expressed as:

$$P = \text{Counts in lipid phase}/\text{Counts in aqueous phase} \quad (2)$$

The amount of salicylate permeating through the epidermis during a sampling interval was calculated based on the measured receptor-phase concentration and volume. The cumulative amount of salicylate permeating per unit area vs time was plotted for each diffusion cell. The steady-state flux was estimated from the slope of the linear portion of the penetration curve. The potential enhancement effect of the addition of the various amines was evaluated by the enhancement ratio, described as the ratio of the permeation rate from the vehicle with amine against that from the vehicle without amine adjuvant.

Table 1. Solubility (Cs) of salicylic acid with or without primary amine counter-ions in various vehicles.

	Cs (mg mL^{-1})		
	Propylene glycol	Toluene	Isopropyl myristate
Salicylic acid	238·4 ± 19·7	5·9 ± 0·2	41·6 ± 0·5
Salicylic acid with:			
Methylamine	220·1 ± 20·8	ND	ND
Ethylamine	269·2 ± 14·7	19·6 ± 0·8	55·5 ± 0·5
Propylamine	278·1 ± 22·6	29·8 ± 0·1	59·7 ± 0·9
Butylamine	278·1 ± 17·4	31·1 ± 0·2	62·0 ± 1·1
Pentylamine	280·5 ± 11·1	32·0 ± 0·2	61·9 ± 0·1
Hexylamine	277·4 ± 13·8	ND	ND
Heptylamine	ND	ND	ND
Octylamine	290·1 ± 21·7	31·5 ± 0·2	63·9 ± 0·9
Nonylamine	ND	ND	ND
Decylamine	ND	ND	ND
Undecylamine	ND	ND	ND
Dodecylamine	282·4 ± 17·4	31·2 ± 0·1	60·9 ± 1·0

ND = not determined

Plasma and tissue samples collected during in-vivo studies were assayed for salicylate using an HPLC method described elsewhere (Megwa et al 1995).

Solute size estimation

These amine counter-ions have molecular weights that are less than 200 Da and their molecular volume (MV) was estimated from partial molal volumes of the fragments comprising the solute (Yalkowsky & Zografi 1972).

Data analysis

All data are reported as mean ± s.e.m., except where indicated. The statistical significance of difference between the groups was determined by analysis of variance and paired *t*-tests. Significance was accepted at the 0·05 level of probability.

Results and Discussion

The solubility (Cs) of salicylic acid in the presence or absence of n-alkylamines in the various vehicles are summarised in Table 1. Salicylic acid is readily soluble in both propylene glycol and isopropyl myristate. None of the amine adjuvants had any great effect on its solubility in these vehicles. The amine adjuvants increased the solubility of salicylic acid in toluene approximately 3–6 fold.

The relationship between the extraction coefficients of salicylate using aqueous solutions and toluene, or 0·5 M amine in toluene over the pH range studied, is shown in Figure 1. These results indicate efficient extraction of salicylate into toluene at low pH values where salicylate is present in its unionised form. The extraction becomes less efficient at high pH since salicylate exists predominantly in its ionised form. The converse will be true of the ionisation state of the counter-ions, pK_a 8·31–11·25, which will be fully ionised at low pH and only partially ionised at high pH. The proportion of salicylate available for ion pairing increased with increasing pH, while there was a reduction in the amount of amine available for ion pairing as the pH increased. This also accounts for the poor extraction of salicylate in the presence of amine at higher pH values. The observed phenomenon is further illustrated in Figure 2. The plot of the ratio of extraction coefficients of salicylate from toluene containing amines to that from

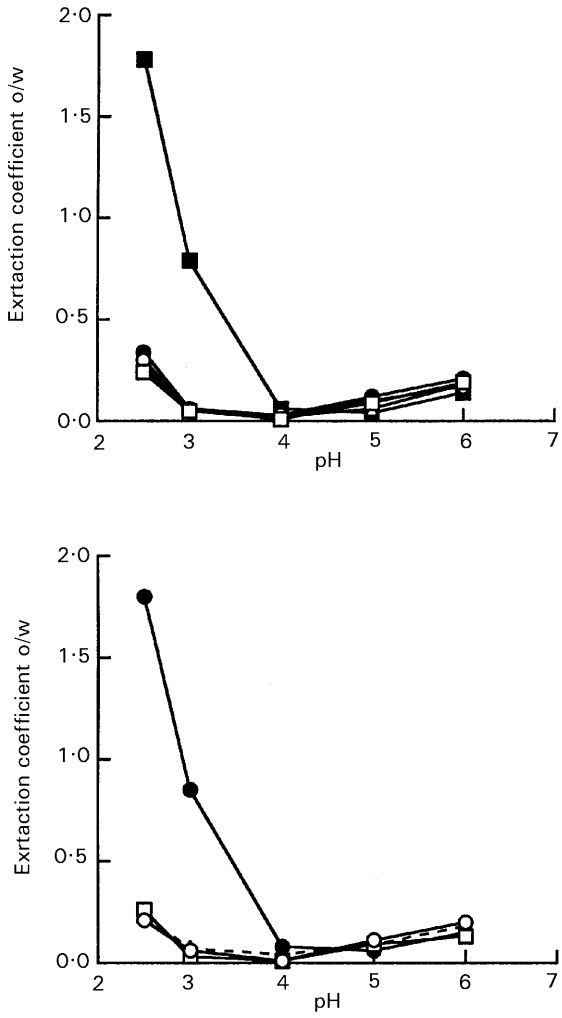

Figure 1. The extraction coefficient vs pH profile of salicylate between aqueous solution and toluene (■) or toluene containing 0·5 M amine. A. Ethylamine (□); diethylamine (○); triethylamine (△); triethanolamine (●). B. Methylamine (●); propylamine (○); butylamine (□); pentylamine (–△–).

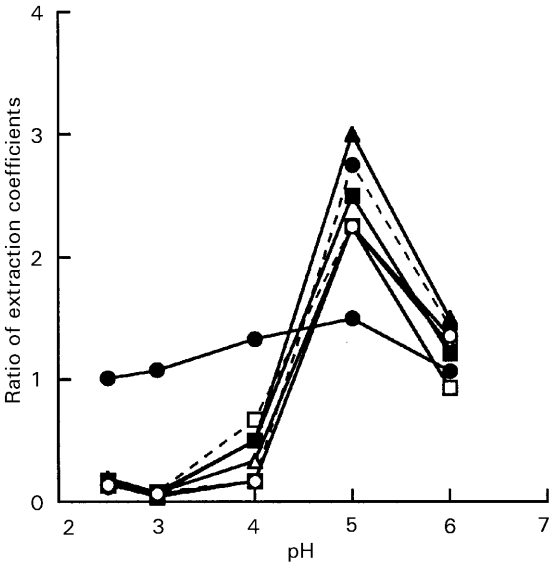

Figure 2. The extraction coefficient ratio vs pH profile for salicylate between aqueous solution and toluene containing 0·5 M amine. Methylamine (●); ethylamine (○); propylamine (- -●- -); butylamine (□); pentylamine (- -□- -); diethylamine (■); triethylamine (△); triethanolamine (▲).

toluene alone against pH gave bell-shaped curves which indicates the possible formation of a more lipophilic compound, an ion pair, between salicylate and alkylamine. The pH/extraction profile for salicylate shows a shift to the right in the presence of amine in toluene and exhibits a maximum peak at pH 5·0 where we propose that the optimum conditions for ion-pair formation between salicylate and amine in this system are met. This behaviour can only be explained on the basis of the ion-pairing mechanism. If the amines are acting as co-solvents, they would remove salicylate at a constant rate and would have been unlikely to be affected by changes in the aqueous pH as observed in this study. The existence of optimum conditions for ion-pair formation observed in this study is in accordance with the result reported by Hadgraft et al (1985). These authors proposed that the optimum conditions for ion-pair formation between salicylate and azone were met at pH 5·5 using 0·1 M azone dissolved in isopropyl myristate as the lipid phase. Green & Hadgraft (1987) also noted that the ratio of the partition coefficient of propranolol hydrochloride into isopropyl myristate plus oleic acid divided by that into isopropyl myristate alone reaches a maximum at around pH 6·0.

Lipophilicity is an important factor in determining the extent of biological activity of compounds (Lee et al 1994). A partition coefficient is nearly always used as a quantitative measure of lipophilicity and is generally measured in a lipid–water system. Partition coefficients (P) of salicylic acid with or without amine are listed in Table 2. The $P_{IPM/water}$ of salicylic acid was increased 4–226 fold in the presence of amines with carbon chain-lengths (C-length) > 5, while $P_{octanol/water}$ increased 3–54 fold in the presence of amines with C-length > 3. These $P_{IPM/water}$ and $P_{octanol/water}$ results are in agreement with the finding of Fruttero et al (1998), who examined the partitioning of homologous (*p*-methylbenzyl)alkylamines in isotropic (octanol–water) and anisotropic (zwitterionic liposome–water) systems and their interactions with zwitterionic liposomes. They observed a constant lipophilicity for short-chain homologues (*N*-methyl to *N*-propyl) in the two systems while there was a steady increase for the long-chain homologues (*N*-butyl to *N*-heptyl). They concluded that the P values for the long-chain homologues mostly expressed hydrophobicity

Table 2. Partition coefficient (P) of salicylic acid with or without amine in various vehicles.

	P				
	IPM/water[a]	Octanol/water[a]	Toluene/PG[b]	Toluene/PG[c]	IPM/PG[c]
Salicylic acid	0·070 ± 0·005	0·550 ± 0·104	0·047 ± 0·004	0·052 ± 0·001	0·291 ± 0·001
Salicylic acid with:					
Methylamine	ND	ND	ND	0·052 ± 0·003	0·270 ± 0·005
Ethylamine	0·040 ± 0·003	0·140 ± 0·004	0·018 ± 0·001	0·045 ± 0·001	0·221 ± 0·007
Propylamine	0·030 ± 0·005	0·340 ± 0·022	0·007 ± 0·001	0·044 ± 0·004	0·241 ± 0·002
Diethylamine	0·020 ± 0·002	0·120 ± 0·016	0·011 ± 0·001	0·046 ± 0·004	0·221 ± 0·003
Butylamine	0·040 ± 0·006	1·710 ± 0·108	0·006 ± 0·001	0·045 ± 0·002	0·204 ± 0·013
Pentylamine	0·090 ± 0·006	5·170 ± 1·350	0·005 ± 0·001	0·043 ± 0·002	0·212 ± 0·007
Triethanolamine	0·040 ± 0·002	0·060 ± 0·006	0·003 ± 0·001	0·043 ± 0·001	0·217 ± 0·002
Triethylamine	ND	ND	ND	0·049 ± 0·003	0·212 ± 0·004
Hexylamine	0·300 ± 0·088	11·220 ± 1·014	0·007 ± 0·001	0·047 ± 0·002	0·212 ± 0·004
Heptylamine	1·640 ± 0·063	17·950 ± 0·138	0·012 ± 0·001	ND	ND
Octylamine	4·700 ± 0·954	24·340 ± 0·658	0·012 ± 0·002	0·047 ± 0·002	0·216 ± 0·002
Nonylamine	8·480 ± 0·970	17·160 ± 0·416	0·019 ± 0·003	ND	ND
Decylamine	14·120 ± 0·962	29·740 ± 4·330	0·024 ± 0·004	ND	ND
Undecylamine	15·820 ± 2·911	22·010 ± 2·344	0·034 ± 0·001	ND	ND
Dodecylamine	12·990 ± 2·092	22·680 ± 3·452	0·045 ± 0·045	0·048 ± 0·003	0·222 ± 0·004

[a]Equimolar concentrations of salicylic acid and amine in phosphate buffer, pH 5·0. [b]Equimolar concentrations of salicylic acid and amine. [c]Salicylic acid and amine are present in 1 : 5 concentration ratio. ND = not determined. IPM = isopropyl myristate. PG = propylene glycol.

while the lipophilicity of the short-chain homologues expressed various electrostatic and polar interactions in the systems. Barbato et al (1996) also proposed that hydrophobicity is the major intermolecular force governing the partitioning of 4-phenyldihydropyridine calcium-channel blockers into biomembranes.

Gustavii (1967) studied the extraction of picrate complexes of homologous series of straight-chain aliphatic primary, secondary and tertiary amines, as well as quaternary ammonium ions, with various solvents. The extraction constants rose with increasing number of methylene groups in the cation and with increasing degree of substitution of the nitrogen. He noted the tendency of other side-reactions such as further association (dimers and tetramers) of the ion pairs in some of the organic phases and stated that the efficiency of extraction depended on both the nature of the solvent and that of the extracted component.

The lack of effect on P in toluene–propylene glycol or isopropyl myristate–propylene glycol systems observed in this study could be due to the degree of solvation of the cation in the aqueous phase and the properties of the ion pairs formed in these systems. Ion-pair transport may be affected by a number of factors (Neubert 1989). For an ionised solute to pass from an aqueous to an organic phase it must initially overcome its hydration energy. The ability of the molecule to remain in the organic phase will be subsequently determined by ion-pair stabilisation within the lipid environment (Green & Hadgraft 1987). It was found that attempts to increase the partitioning of atenolol, the most hydrophilic of the β-blockers studied, through ion-pair formation with both oleic and lauric acids were futile. Ion-pair stabilisation was also not as significant in determining the partitioning behaviour of the highly lipophilic propranolol since it will readily partition into isopropyl myristate at high pH values. The increase in partitioning at high pH values brought about by the addition of fatty acids was more noticeable with metoprolol and oxprenolol which have more balanced partitioning behaviour. Green et al (1989) also noted the importance of balanced partition properties of molecules for their effective permeation.

The strength of the hydrogen bonding occurring in the aqueous phase and the solubility of the ion pairs in the organic phase could also be mitigating factors against the transfer of ion pairs into the lipid phase. Freiser (1969) explained the role of organic phase in promoting ion-pair extraction in terms of the solubility parameters of the formed complexes. Higuchi et al (1967) proposed the specific solvation theory to explain the behaviour of the solvating agent and its affinity for the ion pairs. Based on this theory, ion pairs are classified according to the degree of charge accessibility. In the first case, the ion pair has its anionic charge largely exposed. This system will be most effectively solvated by lipophilic molecules with exposed positively charged surfaces, such as chloroform and phenols. Since the solvating molecules would have their polar end buried adjacent to the anion, the appearance of the

solvated ion pair is that of a relatively non-polar aggregate. In the second case, the cation is largely exposed. Lipophilic molecules, such as ether, ketones and amides which contain nucleophilic sites, would be particularly effective in solvating this type of ion pair. The third case is that of an ion pair with deeply buried charges, thus having no exposed electrically unbalanced surface. Solvation is not necessary for the extraction of the ion pair by non-polar solvent. The solvation theory has been discussed previously (Grant & Higuchi 1990; Quintanar-Guerrero et al 1997).

The cumulative amounts of permeation-time curves for salicylate in the absence or presence of amines permeating the human epidermis are shown in Figure 3. The permeation parameters obtained and the effects of the presence of primary amines on conductivity of ethanol–propylene glycol (2 : 1 v/v) vehicle containing equimolar concentrations of salicylic acid and the amines are shown in Table 3. The permeation parameters of salicylic acid–primary amine ion pairs observed in this study are just slightly lower than those of salicylic acid alone. This is further confirmed by the enhancement ratio values also listed in Table 3. This apparent lack of effect could be due to the formation of solvent-separated or loose ion pairs that lack stabilisation at the interfacial region. This may further result in the formation of ion pairs that dissociate considerably at the interfacial region and thus lack the ability to penetrate the membranes. van der Giesen & Janssen (1982) found evidence that the sodium ion pair of 4-hydroxycoumarin dissociated considerably in the octanol phase. Johansson & Schill (1980) also reported the dissociation of the tetrabutylammonium ion pair of octyl sulphate in 1-pentanol. In both studies, partition coefficients of the ion pairs were found to be slightly lower than those of the undissociated parent compounds.

Another possible explanation for the apparent lack of effect might be due to the formation of salicylic acid–primary amine ion pairs with insufficient lipophilicity to cause an enhancement in the penetration of salicylic acid across the membranes. Neubert & Fischer (1991) studied the effect of lipophilic counter-ions on the transport of ionisable hydrophilic drugs across the dodecanol collodion membrane. They reported that the transport of pholedrine and bretylium was significantly increased by various counter-ions in comparison with the transport of pholedrine sulphate and bretylium tosylate alone. They found that the increase caused by naphthyl derivatives, adamantoate or dehydrocholate was much lower than that caused by salts of fatty acids and hexylsalicylate and concluded that the use of the former ions resulted in the formation of ion pairs with insufficient lipophilicity to achieve transport of these compounds across the membrane.

The in-vitro permeation results obtained in this study contrast with the findings of Kadono et al (1998). These authors reported enhanced in-vitro percutaneous penetration of salicylate through rat and shed snake skins by ion-pair formation with alkylamines of carbon chain-length C_5–C_9. This could be due to the different experimental designs used for the studies. The donor solution in the experiments by Kadono et al (1998) was acetate buffer containing 20-fold molar excess of alkylamine while in our study equimolar concentrations of salicylate and amine in ethanol–propylene glycol (2 : 1 v/v) were used. An objective assessment

Table 3. The conductivity, flux (J) and permeability coefficient (k_p) of salicylic acid and salicylic acid with or without primary amine adjuvants through human epidermis.

	Human epidermis		Conductivity	
	J ($mg\,cm^{-1}\,h^{-1}$) $\times 10^{-1}$	k_p ($cm\,h^{-1}$) $\times 10^{-4}$	Enhancement ratio	($mS\,cm^{-1}$)
Salicylic acid	0·89 ± 1·20	8·9 ± 1·20	1·00	2·03 ± 0·06
Salicylic acid with:				
Methylamine	ND	ND	ND	ND
Ethylamine	0·77 ± 1·20	7·7 ± 1·20	0·87	1·93 ± 0·06
Propylamine	0·75 ± 1·07	7·5 ± 1·07	0·84	2·07 ± 0·06
Butylamine	0·76 ± 1·26	7·6 ± 1·26	0·85	1·93 ± 0·06
Pentylamine	0·75 ± 1·19	7·5 ± 1·19	0·84	2·20 ± 0·00
Hexylamine	0·69 ± 0·77	6·9 ± 0·77	0·78	2·00 ± 0·00
Heptylamine	0·72 ± 0·76	7·2 ± 0·76	0·81	1·93 ± 0·06
Octylamine	0·66 ± 0·78	6·6 ± 0·78	0·74	1·90 ± 0·00
Nonylamine	0·68 ± 0·82	6·8 ± 0·82	0·76	1·80 ± 0·00
Decylamine	0·71 ± 1·02	7·1 ± 1·02	0·80	2·00 ± 0·00
Undecylamine	0·69 ± 0·76	6·9 ± 0·76	0·78	1·70 ± 0·00
Dodecylamine	0·73 ± 0·91	7·3 ± 0·91	0·82	ND

ND = not determined.

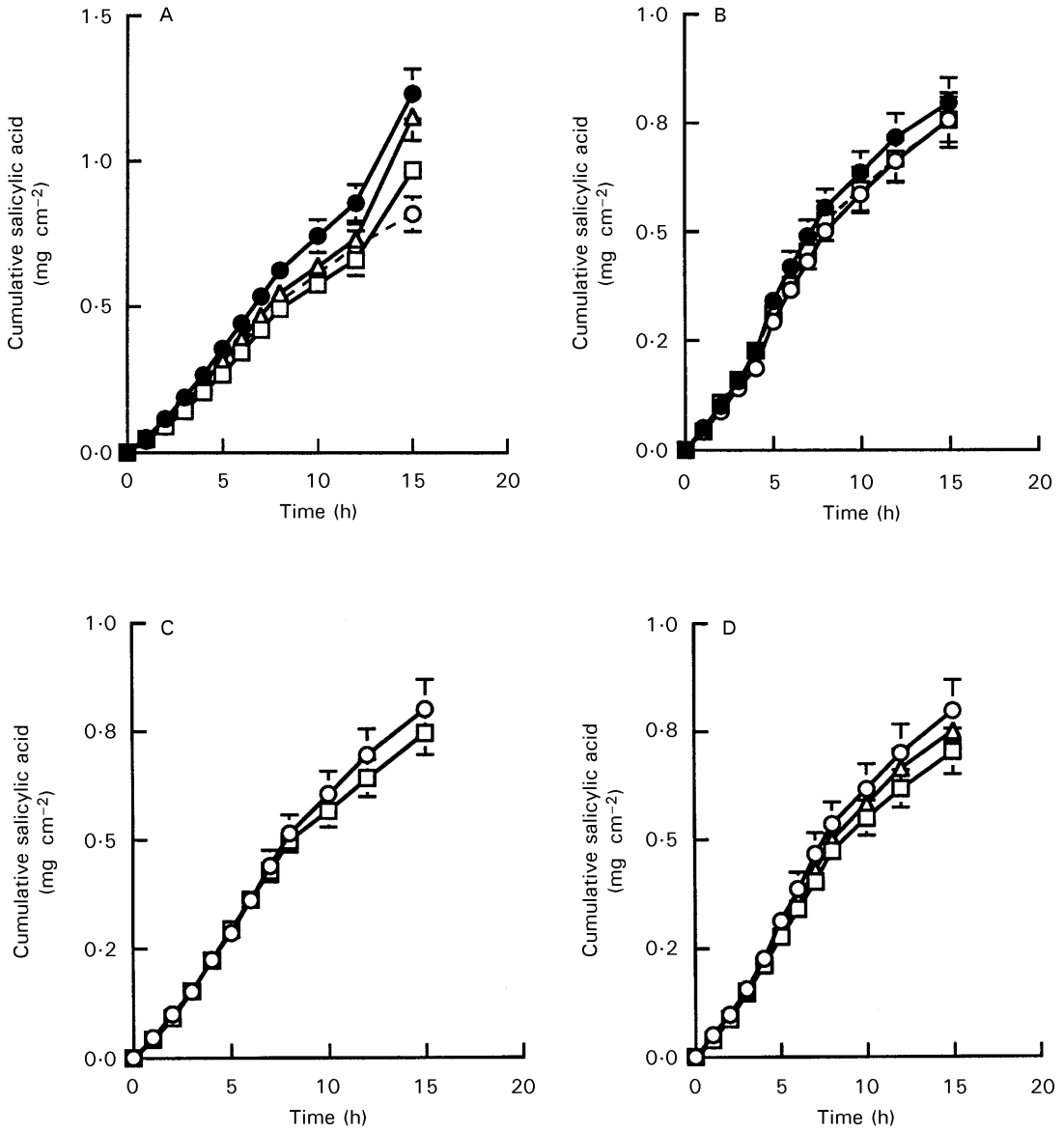

Figure 3. Salicylate concentration permeating human epidermis vs time profile. A. Salicylic acid (●), sodium salicylate (□), salicylate in the presence of ethylamine (△) and propylamine (- -○- -). B. Hexylamine (○), heptylamine (●) and decylamine (- -□- -). C. Butylamine (○) and nonylamine (□). D. Pentylamine (○), octylamine (□) and undecylamine (△).

of the performance of amines as counter-ions in this study could have been obscured by the comparison of equimolar concentrations of salicylate and counter-ions rather than equivalent thermodynamic activities of their respective formulations.

The conductivity of a solution is proportional to the current that is transported through it. The conductivity of a solution containing ionic species is largely dependent on the population of ions present. The ion-pairing process involves charge neutralisation and its formation could thus be observed as a net reduction in the conductivity of the solution where it is occurring. The conductivity of pure vehicle was negligible in all cases ($0{\cdot}23 \pm 0{\cdot}006$, $0{\cdot}33 \pm 0{\cdot}025$ and $0{\cdot}43 \pm 0{\cdot}02\ \mu S\ cm^{-1}$ for ethanol, propylene glycol and ethanol–propylene glycol (2 : 1 v/v), respectively). Similar conductivities were observed for salicylic acid with or without amine in the vehicle (Table 3). It could be inferred from these conductivity measurements that all the salicylate–amine counter-ion solutions used in this study contained identical ionic populations.

The distribution of salicylate in tissues underlying the site of application of the various drug solutions to anaesthetised rats for 6 h, are summarised in Figure 4. Figure 5 shows the plasma salicylate concentration–time profiles from salicylic acid or salicylic acid–amine counter-ion solutions. Salicylate concentrations in the con-

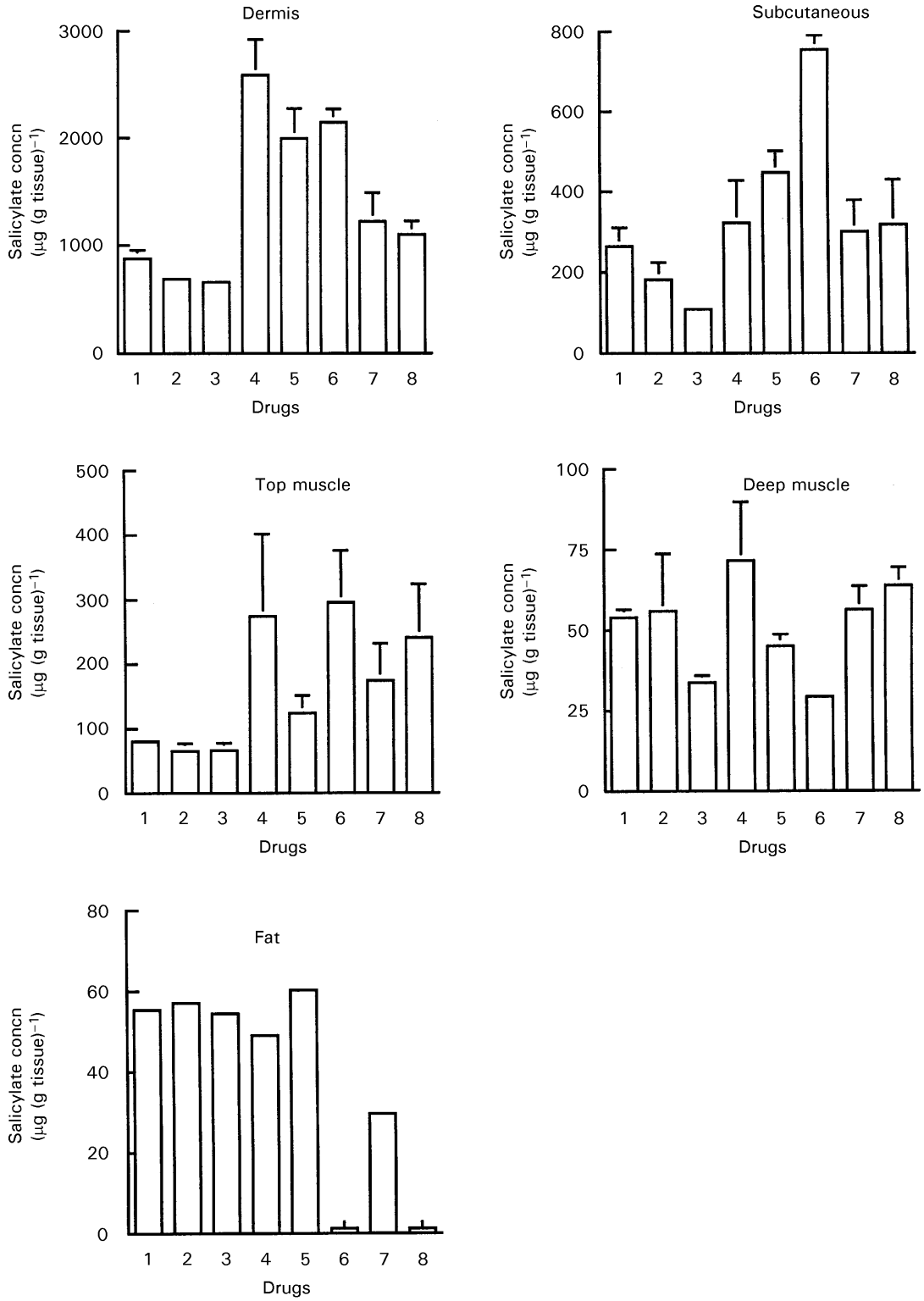

Figure 4. Recovery of drugs from tissues after epidermal application of various drug solutions to anaesthetised rats. 1. Salicylic acid. 2. Sodium salicylate. 3. Salicylic acid–benzydamine. 4. Salicylic acid–ethylamine. 5. Salicylic acid–diethylamine. 6. Salicylic acid–deanol. 7. Salicylic acid–triethanolamine. 8. Salicylic acid–hexylamine.

tralateral tissues were always less than plasma concentrations, consistent with distribution by the systemic blood supply being responsible for the levels of salicylate recovered from these contralateral sites. Similar concentrations of salicylate were recovered from deep muscle and fat after the application of salicylate with or without the amine counter-ions. It could thus be concluded that sali-

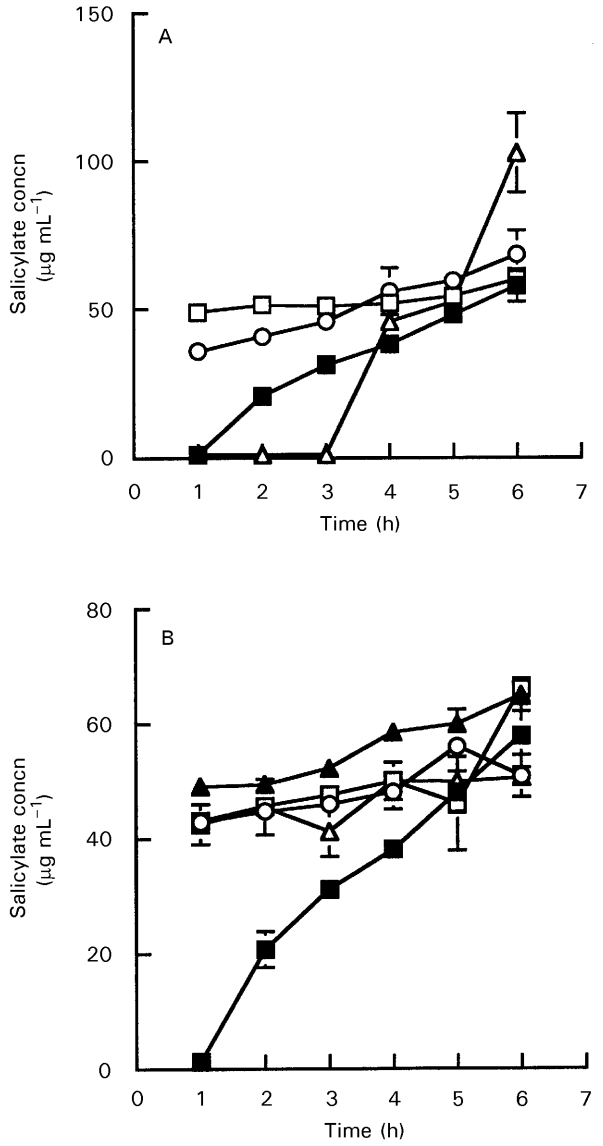

Figure 5. Salicylate recovery from plasma after epidermal application of various drug solutions to anaesthetised rats. A. Salicylic acid (■); sodium salicylate (□); salicylic acid–benzydamine (△); salicylic acid–ethylamine (○). B. Salicylic acid (■); salicylic acid–diethylamine (▲); salicylic acid–deanol (○); salicylic acid–triethanolamine (□); salicylic acid–hexylamine (△).

cylate concentrations in these latter tissues are derived solely from the systemic blood supply.

Although the amine counter-ions seemingly did not affect the delivery rate of salicylate through human epidermis, there is an indication that the presence of counter-ions 4–8 (Figure 4) in the donor solution increased the residency or localisation of salicylate in the dermis, subcutaneous tissues and top muscle. This implies that the rate of clearance of salicylate from these tissues is much lower with the inclusion of counter-ions 4–8 than when salicylic acid alone is administered. Michniak et al (1995) reported that the enhancement of drug delivery can result in three possible outcomes: firstly, enhanced transdermal penetration, applicable particularly for systemic drug delivery; secondly, enhanced skin concentrations of drug, applicable for topical/localised skin-tissue targeting; and thirdly, a combination of these. Co-administering salicylate and counter-ions 4–8 might therefore enhance any local effect of the former in the target tissues. This would support the findings of Hewitt et al (1998), who studied the cutaneous disposition of diclofenac in human skin. They suggested that if the absorbed drug was extracted into the systemic circulation too quickly, concentrations in deeper target tissues (muscle and joints) may be too low to elicit a pharmacological response due to their rapid clearance from these sites.

The plasma salicylate concentration vs time profiles for salicylic acid–amine adjuvant differed from those for salicylic acid alone and sodium salicylate–benzydamine HCl combination. Plasma concentrations of salicylate rose rapidly within 1 h of applying salicylic acid–amine solutions in contrast to the slower distribution rate observed when solutions containing only salicylic acid were applied. The plasma profile for salicylic acid–amine remained fairly constant after the initial peak, suggesting the establishment of a pseudo-equilibrium between plasma and tissues. The enhanced distribution of salicylate observed with salicylic acid–amine solutions within 1 h of their application is consistent with the ability of the amine adjuvants to increase local blood flow, and thus drug clearance, through their local cutaneous irritation effects. This effect of changing cutaneous blood flow on the absorption and uptake of drugs has been previously documented (Danon et al 1986; Heng 1987; Kobayashi et al 1996). Applying the sodium salicylate–benzydamine HCl combination resulted in two distinct peaks in plasma salicylate levels, one at 4 h and a later one at 6 h. This could be due to a change in the partitioning of salicylate into intercellular lipids in the stratum corneum and its binding to cytosol components in the viable skin. The presence of benzydamine could thus change the residence of salicylic acid in the skin. This is in agreement with the finding of Yagi et al (1998), who reported that partitioning of drugs into intercellular lipids and their binding to cytosol components in the viable skin are some of the factors responsible for drug residence or retention in the skin during transdermal absorption.

In conclusion, the results from this study show evidence of ion-pair formation between salicylate and a range of amine counter-ions. It is also apparent that the ability of ion-pair formation to

influence the behaviour of drugs depends strongly on the physicochemical properties of both the drugs and counter-ions. Only a marked change in drug properties upon ion-pair formation can be expected to significantly improve the bioavailability of hydrophilic ionisable drugs. We found that the presence of amine counter-ions had no marked effect on the in-vitro penetration of salicylate through human epidermis. However, there appeared to be an increase in residency and localisation of the drug in tissues in-vivo, which implied a lower systemic extraction. It is therefore apparent that studying the in-vitro penetration of salicylate in the presence of amine counter-ions does not necessarily predict the efficiency of salicylate uptake in the in-vivo transdermal delivery in rat model. While ion-pair formation could be a valuable tool for the lipophilisation of ionic drugs for transport through the skin, the search for specific lipophilic counter-ions (with appropriate physiological compatibility) is still necessary to fully exploit the benefits of ion-pair transport of drugs through biological membranes.

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