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# Permeability of human epidermis to phenolic compounds

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The permeability of human epidermis to many phenolic compounds can be related to octanol/water partition coefficients. For a series of compounds having similar diffusion coefficients and showing an increase in lipophilic character, the maximum flux will be obtained with the less lipophilic compounds because the solubility decreases more rapidly than the partition coefficient increases. The penetration of some phenolic compounds is concentration dependent, a constant permeability coefficient being observed below a threshhold concentration above which the permeability coefficient increases with concentration. The increase in the permeability coefficient is attributed to 'damage' to the epidermis and a reduction in its diffusional resistance. A relation between the ability of a substance to damage the skin and the lipophilic character (or more generally the penetration flux) of the substance was found. A number of substances do not appear to 'damage' the skin due to insufficient substance partitioning or penetrating into the stratum corneum. This 'cut-off' in effect is generally observed for the more lipophilic substances. For a series of compounds, maximum damage will be obtained with the less lipophilic compounds in accordance with the predicted concentrations of the substance in the stratum corneum. Phenolic compounds appear to interact with the skin by protein denaturation although for the more lipophilic compounds some degree of 'plasticization' of the lipids is evident.

Phenolic compounds are widely used in topical preparations for their local anaesthetic, antipruritic or antibacterial properties; they are generally applied to the skin either as preservatives or to obtain a local effect. Under certain conditions phenol is known to damage skin leading to increased penetration rates (Roberts, Shorey & others, 1974; Roberts & Anderson, 1975).

This paper examines the influence of concentration and structure of various phenolic compounds on the permeability of human skin.

## MATERIALS AND METHODS

### *Materials*

The cresols, chlorophenols and phenol were redistilled before use. Chlorocresol, methyl hydroxybenzoate, resorcinol and thymol (B.P.), chloroxylenol (B.P.C.) and other compounds (reagent grade) were used as received. Glass distilled water was used. Epidermal membranes were separated from human abdominal skin obtained at autopsy by exposure to ammonia fumes for 30 min (Kligman & Christophers, 1963).

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### *Analysis*

Spectrophotometric analyses were made at the wavelength of maximum absorption for the particular phenolic compound.

### *Solubility determinations*

Excess solutes were equilibrated with water in stoppered glass containers at about 50°. The containers were placed in a shaker bath at 25° and agitated gently for approximately one week. Precipitation of solute from the solution was indicative of saturation. Samples of solution were removed via glass wool-tipped pipettes and appropriately diluted for analysis.

### *Permeability studies*

Pyrex glass cells (Roberts & others, 1974) were used with a membrane, of exposed area 2·5 $cm^2$, supported between the two halves of the cell according to Scheuplein (1965) except that the membrane was protected by a wire mesh and a disc of filter paper (Whatman No. 1). A watertight seal was made with Apiezon T spread on the lapped glass surfaces; the edges of the filter paper were impregnated with hard paraffin. The compartments were each of 30 ml but volumes as low as 9 ml could be used. The receptor side (and when necessary the donor side) was stirred by an air driven paddle. Samples withdrawn from the receptor side were

replaced by either the original sample or an equivalent amount of distilled water. Concentrations in the receptor compartment were low compared to those in the donor compartment and were considered negligible. All studies were made at least in duplicate at 25 ± 0·1° unless otherwise stated.

To ensure reproducibility, skin samples from one area of one subject were used for each series of experiments. If necessary each membrane was used for several experiments. Its integrity was examined at the end of each series by repeating the initial experiment and comparing the fluxes obtained.

Penetration fluxes and permeability coefficients ($k_p$) were estimated from the steady state slopes of the relations between the cumulative amount of solute penetrating through unit area of membrane with time, in accordance with the equation:

$$J = Q/At = KDC_v/h = k_pC_v \quad .. \quad (1)$$

where J is the molecular flux of the solute, Q is the amount of solute which diffuses through area A in time t, K is the solute's stratum corneum/vehicle partition coefficient, D is the diffusion coefficient of the solute in the stratum corneum of thickness h, $C_v$ is the concentration of the solute in the vehicle, and $k_p$ ($= DK/h = J_s/C_v$) is the permeability coefficient (Scheuplein & Blank, 1971).

The threshold concentration for damage (the aqueous concentration at which the $k_p$ value begins to increase) was estimated graphically. The extent of damage may be indicated by the ratio of $k_p$ calculated for a damaging concentration to that for non-damaging concentrations of the solute. The reversibility of this damage has been examined by comparing the $k_p$ values of fluxes of solutes before and after treatment with a damaging concentration of solute for 3 h. (The fluxes after damage were measured following desorption of residual solute.)

## RESULTS AND DISCUSSION

Fig. 1 shows plots of cumulative amounts of chlorocresol and resorcinol penetrating through epidermis against time. After a lag period, linear relations were observed giving a constant $k_p$. For higher concentrations of some solutes, the steady-state $k_p$ increases as a result of damage to the stratum corneum (see later). The $k_p$ values from non-damaging concentrations of a range of phenolic compounds are listed in Table 1.

The lag times of phenolic compounds differ markedly (Table 2). Phenol, the cresols and bromophenol have lag times of about 15 min while those for β-naphthol, *m*-nitrophenol and resorcinol are greater by factors of about 2, 3 and 5 respectively. The addition of polar groups to a solute decreases its diffusion coefficient (D) in the stratum corneum (Scheuplein, Blank & others, 1969; Scheuplein & Blank, 1971) and the longer lag times may be attributed to this effect.

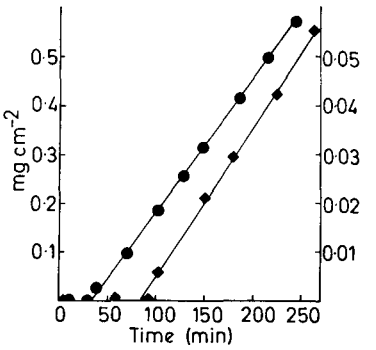


FIG. 1. Penetration of chlorocresol and resorcinol through human epidermis from aqueous solutions. ●—chlorocresol 0·4% w/v (left scale). ◆—resorcinol 10% w/v (right scale). Ordinates—Cumulative amount of solute penetrated mg cm$^{-2}$).

Table 1. *Permeability coefficients (I, cm min$^{-1}$ × 10$^4$), threshold concentrations for damage† (II), solubility (III) and partition date for various phenolic compounds.*

| Solute | I | II (% w/v) | III (% w/v) | $\log_{10}$P* |
|---|---|---|---|---|
| Resorcinol | 0·04 | n** | | 0·8 |
| *p*-Nitrophenol | 0·93 | 0·9 | 1·4 | 1·96 |
| *m*-Nitrophenol | 0·94 | 0·8 | 1·3 | 2·00 |
| Phenol | 1·37 | 1·5 | 7·8 | 1·46 |
| Methyl hydroxybenzoate | 1·52 | n | 0·2 | 1·96 |
| *m*-Cresol | 2·54 | 1·0 | 2·5 | 1·96 |
| *o*-Cresol | 2·62 | 0·9 | 2·5 | 1·95 |
| *p*-Cresol | 2·92 | 8·85 | 2·1 | 1·95 |
| β-Naphthol | 4·65 | n | 0·1 | 2·84 |
| *o*-Chlorophenol | 5·51 | 0·8 | 2·2 | 2·15 |
| *p*-Ethylphenol | 5·81 | n | 0·5 | 2·40 |
| 3,4-Xylenol | 6·00 | n | 0·5 | 2·35 |
| *p*-Bromophenol | 6·02 | 0·95 | 1·5 | 2·59 |
| *p*-Chlorophenol | 6·05 | 0·75 | 2·4 | 2·39 |
| Thymol | 8·80 | n | 0·1 | 3·34 |
| Chlorocresol | 9·16 | n | 0·5 | 3·10 |
| Chloroxylenol | 9·84 | n | 0·03 | 3·39 |
| 2,4,6-Trichlorophenol | 9·90 | n | 0·09 | 3·69 |
| 2,4-Dichlorophenol | 10·01 | n | 0·5 | 3·08 |

* P is the octanol-water partition coefficient of solute (from data of Hansch, 1971; Leo, Hansch & Elkins, 1971; Davis & others, 1974).

† n indicates no damage observed for any concentration of solute up to saturation; n** indicates no damage up to 40% w/v.

Table 2. *Lag times from permeability experiments.*

| Solute | Concentration (% w/v) | Time lag (min) |
|---|---|---|
| Phenol | 0·4 | 15 |
| *o*-Cresol | 0·4 | 15 |
| *m*-Cresol | 0·4 | 15 |
| *p*-Bromophenol | 0·4 | 15 |
| *p*-Cresol | 0·4 | 16 |
| 3,4-Xylenol | 0·2 | 16 |
| Chlorocresol | 0·4 | 17 |
| Chloroxylenol | 0·01 | 18 |
| Thymol | 0·10 | 18 |
| β-Naphthol | 0·05 | 30 |
| *m*-Nitrophenol | 0·5 | 50 |
| Resorcinol | 10 | 80 |

Insignificant amounts of resorcinol and chlorocresol penetrated the skin in the time before the establishment of steady-state permeation (Fig. 1). The contribution of shunt diffusion to the overall penetration process will, therefore, be small; the lag period for shunt diffusion is only a few seconds (Scheuplein & Blank, 1971) whereas that for steady-state penetration is between 10 and 100 min.

*Partition coefficient*

The partition coefficient is a factor in the penetration of a wide variety of solutes through biological membranes (Lien, 1975). Fig. 2 shows the Hansch-type relation between the $k_p$ values of phenols and their octanol/water partition coefficients (P). Resorcinol, methyl hydroxybenzoate, *m*-nitrophenol and *p*-nitrophenol have reduced D values because of the presence of additional polar groups (Anderson, Triggs & Roberts, 1976). Consequently, these deviate from the general linear relation and have not been included in Fig. 2. To show the relation

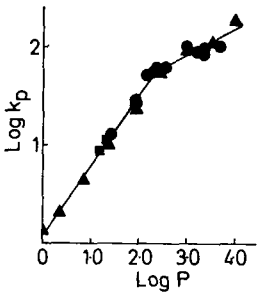


FIG. 2. Relation between the permeability coefficients ($k_p$) of solutions through human epidermis from aqueous solutions and the solutes' octanol-water partition coefficients (P). ●—phenolic compounds (from Table 1). ■-aromatic alcohols (Roberts, 1976). ▲—aliphatic alcohols (data from Scheuplein & Blank, 1973). Ordinate—Log ($k_p \times 10^5$ cm min$^{-1}$).

of $k_p$ with a wider range of P values, the permeability data for alcohols have been included.

The linear portion of the plot of log $k_p$ against P value has a slope of about 0·6 (Fig. 2). A similar slope is found when log stratum corneum/water partition coefficients are plotted against those for octanol/water (Roberts, Triggs & Anderson, 1975) confirming that the transepidermal pathway is the main avenue of penetration for phenolic compounds. If shunt diffusion was a significant pathway then the slope of the relationship between log $k_p$ and log P should be less than that between log K and log P; the slope between log $k_p$ and log P will be zero when shunt diffusion is dominant. The deviation from linearity in Fig. 2 for higher P values is consistent with the parabolic relation observed for a number of biological systems (Hansch, 1971; Davis, Higuchi & Rytting, 1974).

*Maximum flux of phenols across intact skin*

The penetration flux of solute across the stratum corneum is related to the concentration ($C_m$) of solute in that side of the membrane to which the solute is applied (Scheuplein & Blank, 1971) and is given by

$$J = DC_m/h \quad .. \quad .. \quad .. \quad (2)$$

Many phenolic compounds have similar diffusion coefficients in stratum corneum (Anderson & others, 1976), and in such a series the maximum flux will be directly related to the solubility of the solute in the membrane.

The molar solubility of the solute in the stratum corneum, $S_m$, will be related to the molar solubility of the solute in the vehicle, $S_v$, by

$$S_m = KS_v \quad .. \quad .. \quad (3)$$

providing the stratum corneum/vehicle partition coefficient (K) is constant for all concentrations up to saturation. For an aqueous system, equation (3) can be written

$$S_m = KS_{aq} \quad .. \quad .. \quad .. \quad (4)$$

or

$$\log S_m = \log K + \log S_{aq} \quad .. \quad .. \quad (5)$$

where $S_{aq}$ is the molar solubility of the solute in water.

Consequently, the extent to which K and $S_v$ vary with structural modification of the solute will govern the likely solubility of the solute in the stratum corneum and, hence, the maximal flux. In an

homologous series of solutes, each methylene group causes an increase of about 0·3 in log K (Scheuplein & Blank, 1971; Roberts, 1976) and a decrease of 0·6 in log $S_{aq}$ (from data in Table 1), so that log $S_m$ will decrease by 0·3. Consequently, the longer chain members of an homologous series will have lower concentrations of solute in the stratum corneum ($S_m$) following equilibration with their saturated aqueous solutions than other members. Since the log $S_{aq}$ of most non-electrolytes (e.g. alkyl halides, alcohols, acids, esters) decreased by 0·4–0·7 on the addition of each methylene group (Saracco & Spaccamela Machetti, 1958), a decrease in maximal flux with an increase in the chain length of solutes is likely to be a general phenomenon.

The maximum flux may be estimated as the product of the aqueous molar solubility of a solute and the $k_p$ value for non-damaging concentrations (Table 1). The relation between the estimated maximum fluxes of phenolic compounds and their P value is shown in Fig. 3; it can be seen that the maximum fluxes for the halogen-substituted phenolic compounds are substantially greater than those estimated for other phenolic compounds with similar P values.

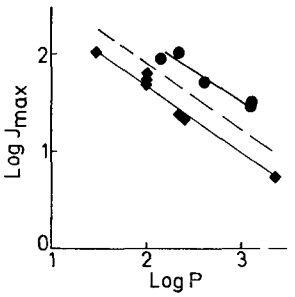


FIG. 3. Relation between $J_{max}$, the estimated maximum flux for phenolic compounds through human epidermis and P, their octanol-water partition coefficients. ●—halogenated phenolic compounds. ◆—nonhalogenated phenolic compounds. — — predicted slope. Ordinate—Log ($J_{max}$ × $10^5$ mol cm$^{-2}$ min$^{-1}$).

The observation that the addition of a methylene group will decrease the solubility of a solute in the stratum corneum appears to be generally valid. Scheuplein & Blank (1973) found that the penetration flux of various alcohols as pure substances decreased with the addition of each methylene group (from methanol to octanol); and, for a series of alkyl phosphates, applied as solvent-deposited solids, Marzulli, Brown & Maibach (1969) found penetration rates to decrease with increase in the benzene water partition coefficient.

*Damaging concentrations*

The influence of phenol concentration on permeation through stratum corneum is shown in Fig. 4. After an initial lag, a constant flux is observed for at least 8 h. The flux ($J_s$) of phenol depends on concentration ($C_v$) and increases markedly for high concentrations (Fig. 5).

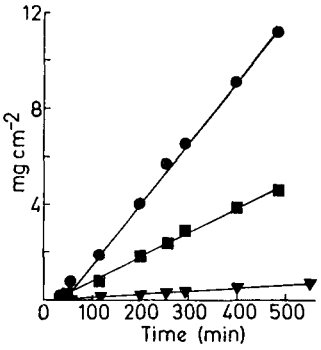


FIG. 4. Penetration of phenol through human epidermis from various aqueous concentrations. ▼ 1%, ■ 4%, ● 7%. Ordinate—cumulative amount of phenol penetrated (mg cm$^{-2}$).

Similar results were obtained for several other phenols. For dilute solutions (less than about 1%), the $k_p$ value is constant showing that penetration from these solutions proceeds according to Fick's law. Above a threshold concentration, the $k_p$ value for some phenolic compounds increases with concentration (Fig. 5). The permeability coefficients in dilute solutions and the threshold concentrations for these compounds are included in Table 1.

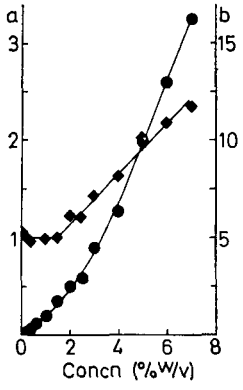


FIG. 5. Effect of concentration of phenol on its penetration through human epidermis from aqueous solutions. ◆—permeability coefficient. ●—penetration flux. Ordinates (a) Permeability coefficient (cm min$^{-1}$ × $10^4$). (b) Penetration flux (mg cm$^{-2}$ min$^{-1}$ × $10^3$).

It is suggested that the increased permeability of the stratum corneum above the threshold concentration is caused by damage to the membrane, and not by increased activity coefficients for the solutes in the higher concentrations. This conclusion is supported by findings that there is no increase in $k_p$ at higher concentrations of phenol for permeation through a polyethylene membrane and that the partition coefficient of phenol between arachis oil and water is independent of concentration up to saturation (Roberts, 1976). The differences in flux of phenol from low concentrations of phenol through excised skin before and after treatment with damaging concentrations (Table 3) confirms this

Table 3. *Comparison of permeability coefficients ($k_p$) of penetrant from a dilute aqueous solution following contact with a concentrated solution.*

| Solute concn used to cause damage | $k_p$ (cm min$^{-1}$ × 10$^4$) Dilute soln | Concn soln | Dilute soln after concn soln |
|---|---|---|---|
| Phenol (5% w/v) | 1·1 | 2·1 | 2·4 |
| Phenol (s.s.) | 1·1 | 3·3 | 3·5 |
| *p*-Nitrophenol (s.s.) | 1·2 | 1·8 | 1·8 |
| *m*-Cresol (s.s.) | 2·0 | 3·7 | 3·4 |
| *o*-Chlorophenol (s.s.) | 3·2 | 5·0 | 3·8 |
| *p*-Chlorophenol (s.s.) | 3·5 | 5·3 | 4·0 |
| *p*-Bromophenol (s.s.) | 5·6 | 8·4 | 7·0 |

s.s.—saturated solution.

effect. These results are consistent with the observation of Deichman (1949) that the local effects of phenol on the skin are in direct proportion to the concentration of the solution.

Fig. 6 illustrates the effect of temperature on the $k_p$ value of phenol from various aqueous concentrations. The activation energies for permeation are comparable for 0·5 and 1% solutions of phenol, but for higher concentrations, activation energies for permeation are reduced. This also suggests that higher concentrations of phenol reduce the resistance of the skin to penetration. Scheuplein & Ross (1970) observed that the activation energy for diffusion of water was markedly reduced on delipidization of stratum corneum membranes, and suggested that this treatment results in a fairly porous, non-selective membrane with the low activation energy indicating diffusion through water-filled channels.

*Structure-activity relations*

The Hansch approach has been used to correlate physicochemical properties of a solute and biological response and is applicable to structurally non-specific action (Hansch, 1971). The interaction of phenols with the skin appears to be a physical toxicity phenomenon where the concentration of phenolic compound in the stratum corneum determines the extent of damage. Fig. 7 shows the Hansch-type relation between molar threshold concentration of the phenolic compound and its P value (data from Table 1). Solutes with P values greater than that of *p*-bromophenol (log P = 2·59) did not cause damage. In the same way that equation (5) was used earlier to estimate maximum flux, it can now be argued that some solutes have an insufficient aqueous concentration (solubility) to reach a threshold concentration in the stratum corneum. Insufficient solute is partitioned into the stratum corneum to produce denaturation of the keratin. A similar 'cut-off' has been observed in many biological systems (Ferguson, 1939) and is found, in ascending an homologous series, when the solubility decreases more rapidly than the partition coefficient increases. The relation between the molecular structure of surface-active agents and

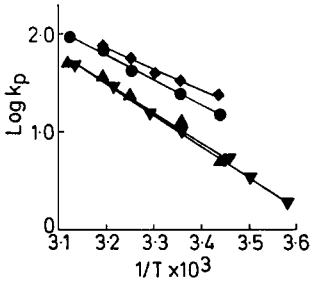


FIG. 6. Arrhenius-type plots between the penetration of phenol through human epidermis from various aqueous concentrations and temperature. ▼ 0·5%, ▲ 1·0%, ● 4·0%, ◆ 7%. Ordinate—Log ($k_p$ × 10$^5$ cm min$^{-1}$).

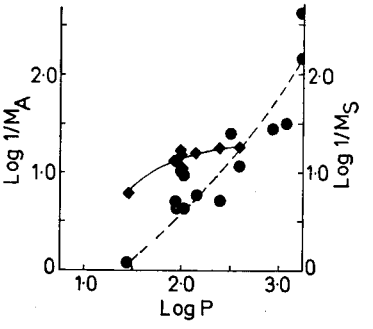


FIG. 7. Relation between P, the octanol-water partition coefficient, $M_A$, the molar threshold concentration (◆) and $M_S$, the molar solubility (●). Data from Table 1.

their effects on skin permeability shown by Dugard & Scheuplein (1973) appears to conform to this hypothesis.

In an homologous series the logarithm of certain properties (e.g. vapour pressure, solubility, partition coefficient, toxic concentration) is linearly related to the number of carbon atoms (Ferguson, 1939; Scheuplein & Blank, 1971; Davis & others, 1974). Such a relation also holds for the extent of damage by the saturated solutions of some phenolic compounds.

Thus the logarithm of the extent of damage for phenols decreases by approximately 0·2 for each methylene group. This is the same order of decrease as predicted for log $S_m$ and indicates that the extent of damage is dependent on the concentration of solute in the stratum corneum, and consequently, the penetration flux (eqn 1).

For other phenolic compounds, the extent of damage is probably related to both concentration and the diffusivity of the solute in the stratum corneum, the addition of polar groups to a solute considerably reducing the extent of damage. Damage produced by phenol appeared to be substantially greater than that observed for resorcinol, as evidenced by histological changes (Roberts, 1976) and permeability studies (Table 1).

The effects of other solutes on the stratum corneum also confirm the importance of the number of polar groups in a solute and the extent of damage. For example, ether increases the permeability of the epidermis to a greater extent than ethanol, whereas acetone gives intermediate changes in permeabilities (Scheuplein & Ross, 1970). Consequently, the extent of damage is probably dependent on both the concentration of solute in the stratum corneum and its diffusion coefficient, i.e. it is likely that the extent of damage is dependent on the penetration flux of a solute. It is evident that the maximum flux of some phenolic compounds is insufficient to produce damage. Consequently, whereas some phenolic compounds such as phenol, cresols and chlorophenols are caustic to the skin, others produce little or no damage (chlorocresol, chloroxylenol, thymol, hexachlorophane).

This relation between damage and the penetration flux appears to exist for other series of substances. Allenby, Fletcher & others (1969) reported that the damage for pure aliphatic carboxylic acids decreased with an increase in the molecular weight of the acid. Because molecular weight increases are synonymous with increases in lipophilic character for solutes with the same number of polar groups, these results are consistent with the proposed relationship between damage and flux. Scheuplein & Blank (1973) have reported similar results for aliphatic alcohols, greatest damage being observed for methanol and progressively less damage for ethanol and propanol; other alcohols (butanol to octanol) do not appear to damage the stratum corneum to any significant extent implying that a 'cut-off' in ability to damage exists in this series.

However, the extent of damage is also likely to depend on the mechanism involved. Thus, examination of the surface changes produced in skin by benzene and phenol (Roberts, 1976) indicates that these substances interact with skin by different mechanisms. Consequently, the relation between damage and flux is probably only valid for substances affecting the integrity of skin by similar mechanisms.

*Mechanism of damage*

Aqueous solutions of phenolic compounds probably affect the integrity of the skin by protein denaturation; such a mechanism has been proposed for phenol by Rothman (1964). The proposed model of protein denaturation in which an equilibrium exists between native and denatured protein (Tanford, 1968) suggests that interaction of a solute with native protein produces a protein-solute complex and that a sufficient concentration of this complex is required to produce denaturation. To cause this type of effect, a threshold concentration of a phenol in the stratum corneum would be required; higher concentrations of phenols would result in increased proportions of complex, more denaturation and hence greater permeability of the membrane.

It is also probable that the more lipophilic phenols plasticize the lipids of the stratum corneum. Scheuplein & Ross (1970) have suggested plasticization as a possible explanation for the ability of some non-polar alcohols to enhance absorption of solutes. This effect is consistent with the reversibility of damage observed for the more lipophilic phenolic compounds (Table 3).

Toxicity of solutes *in vivo* may be influenced by factors additional to those observed with excised skin (Roberts & others, 1974) and may arise from systemic absorption, dermal absorption or changes to the epidermis following contact with various substances. In this *in vitro* study the extent of damage has been measured in the steady-state when damage is complete. The time of contact before this is achieved can vary from 15 min to 5 h and generally the rate at which damage occurs is greatest

for those solutes producing the maximum damage (Allenby & others, 1969; Scheuplein & Blank, 1973; Roberts & others, 1974). The extent of toxicity will also be affected by the choice of vehicle, the cutaneous blood supply and the area of contact (Roberts & others, 1974; Roberts & Anderson, 1975).

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