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Effect of Perfusion Flow Rate on the Tissue Uptake of Solutes After Dermal Application Using the Rat Isolated Perfused Hindlimb Preparation

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Abstract—The rat perfused hindlimb preparation was investigated as a model to study the dermal clearance and tissue distribution of three solutes with differing physicochemical properties ([^{3}H]water, [^{3}H]diazepam, and [^{14}C]lignocaine). The dermal clearance of each solute increased and the depth of tissue preparation decreased with increasing perfusion flow rate. The half-life for solute efflux from tissues into perfusate, and the peak times for amount of solute in tissue were in the order water > lignocaine > diazepam. Topical iontophoresis, which has previously been shown to achieve similar clearances from topical sites to those seen following passive dermal application, resulted in a clearance of lignocaine in the perfused hindlimb comparable with that obtained in the preparation following passive absorption through exposed dermis. The distribution of capillary blood flow in the perfused hindlimb was quantified using ^{51}Cr-labelled 15-μm microspheres, with greater increases in flow found in deeper muscle tissues with increasing perfusion flow rate compared with superficial layers. Microsphere studies in anaesthetized rats also demonstrate slight differences in the distribution of flow within the hindlimb compared with the perfused preparation. We conclude that the rat perfused hindlimb preparation successfully combines many of the advantages of in-vitro and in-vivo methods and has potential for routine use in the study of solute dermal absorption kinetics.

In-vitro absorption studies, using postmortem skin or model membranes, have provided valuable information on the kinetics of solute skin penetration. In-vitro diffusion cells have advantages for accurate determinations of solute absorption rates since frequent sampling from beneath a fixed skin surface area can be achieved. However, in-vitro models do not provide any information about the role of blood flow in solute clearance from a particular topical application site, or the subsequent tissue distribution of a solute following skin penetration. In-vivo animal studies have the advantage of providing absorption sites of living skin, with a viable blood supply, and underlying tissues for dissection and subsequent solute concentration determinations. However, in-vivo models, particularly those using anaesthetized animals, present other complications such as maintaining constant conditions of blood flow, and quantifying the amount and depth to which a solute is absorbed with minimal inter-animal variation.

The importance of tissue blood flow on solute absorption has been recognized for intestinal absorption for some time (Winne & Remischovsky 1970) and has more recently been implicated as a major determinant of dermal absorption rates (Siddiqui et al 1985, 1989; Riviere et al 1992; Singh & Roberts 1993a). Isolated perfused tissue models with a viable vascular system have been developed for studying percutaneous penetration, these have included the isolated porcine skin flap (Carver et al 1989), bovine udder (Kietzmann et al 1991), rabbit ear (Behrendt & Kampffmeyer 1989) and dog leg (Pershing & Krueger 1987). The use of perfused isolated tissues allows incorporation of the effects of living processes into percutaneous absorption kinetics; however, in considering their application to routine laboratory studies the existing models tend to use the more expensive laboratory animal species or involve the collection and transport of tissues of slaughtered animals. We have therefore investigated the use of the rat perfused hindlimb as an alternative isolated in-situ model for the study of percutaneous absorption.

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The rat perfused hindlimb provides ample skin surface area for the positioning of diffusion cells, to epidermal, dermal, and subcutaneous sites, a good depth of peripheral tissue beneath absorption sites for determination of solute penetration, ease of unlimited perfusate samplings, and accurate control over the rate of tissue perfusion throughout the study period. We report the rate of absorption and subsequent tissue distribution of three model solutes, chosen for their differing protein binding properties, degrees of ionization at physiological pH and molecular size. Studies include passive dermal delivery from diffusion cells at different perfusion flow rates, together with quantification of distribution into underlying tissues, topical application by iontophoresis and analysis of the effect of some solute physical properties on absorption profiles.

Materials and Methods

Rat hindlimb perfusions

Hindlimb perfusions were established in a similar manner to that described elsewhere (Cote et al 1985; Colquhoun et al 1988; Clark et al 1990). Briefly, male Wistar rats, 280–340 g, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), the abdomen opened and the right femoral artery cannulated (PE50) via the aorta. A second cannula (PE205)

was placed in the dorsal vena cava, and the hindlimb perfused in a humidicrib at 37°C with oxygenated (95%O_2–5% CO_2) Krebs–Heinseleit–Ringer (mM: NaCl 118, KCl 4·74, KH_2PO_4 1·18, $MgSO_4 \cdot 7H_2O$ 1·18, $CaCl_2 \cdot 2H_2O$ 2·54, $NaHCO_3$ 25, glucose 11·1; pH 7·4, 37°C), containing 4% bovine serum albumin (Fraction V, Sigma). Once the perfusions were established, rats were killed by creating a pneumothorax and turned over so that cells could be attached to the outer thigh of the perfused limb. The pressure of the inflowing perfusate was continuously monitored, mean pressures were in the order of 30 and 40 mmHg at 4 and 8 mL min^{-1}, respectively. The perfused preparation is stable for up to 2 h (Colquhoun et al 1988; Wattel et al 1990), as validated by testing inflowing and outflowing perfusate samples for pH, dissolved oxygen, and various enzyme and ion concentrations (Wu et al 1993). The perfusion flow rates were controlled by a graduated peristaltic pump, and were measured at the beginning and end of each experiment.

Passive dermal absorption studies

Glass diffusion cells (8 cm high, 1.8 cm int. diam., 2·54 cm^2 skin application area), identical to those used for our in-vivo dermal penetration studies (Singh & Roberts 1990) were fixed to the skin on the outer thigh of the hindlimb with adhesive. The application area on the hindlimb was first depilated with commercial Nair hair-removal cream and the stratum corneum removed, where required, using a dermatome before the perfusion was established. Solutions (2 mL) containing trace amounts of the radiolabelled solute of interest ([^{3}H]water (Sigma Chemical Co., Australia), [^{3}H]diazepam (Amersham, Australia) or [^{14}C]lignocaine (NEN, c/o Dupont, Australia)) in phosphate buffered saline (pH 7·4, 37°C) were introduced into the cell at time 0, and 10-μL samples removed at 15-min intervals through a sampling port. Absorption studies were conducted for 90 min, after which time the cell contents were drawn out with a pipette, the cell removed and the tissues from beneath the position of the cell sequentially dissected into preweighed vials for weight determination, solubilization and liquid scintillation counting. The clearance (CL, mL min^{-1}) of solutes from the cell was determined from the relationship:

$$CL = k_a \cdot V \quad (1)$$

where k_a is the disappearance rate constant (min^{-1}) calculated from the relationship between the logarithm of the percentage of solute remaining in solution with time (min) and V is the volume applied (mL) (Siddiqui et al 1985).

Samples of outflowing perfusate were collected from the venous catheter and analysed for solute concentration. The cumulative amount of solute eluted in the perfusate (M_{eluted}) at various times was calculated from the product of perfusate flow rate (Q_p) and the cumulative AUC (area under the curve) of the plot of concentration in the perfusate against time (min) using the trapezoidal rule:

$$M_{eluted} = \int_0^t Q_p \cdot C\,dt = Q_p \cdot AUC \quad (2)$$

The total amount of solute in the tissues (M_{tiss}) at time t, was estimated from:

$$M_{tiss} = M_{cell} - M_{eluted} \quad (3)$$

where M_{cell} is the amount of solute lost from the absorption cell at time t.

Elimination rate constants (k_e) were estimated from the cumulative amount of solute in the outflowing perfusate assuming the hindlimb behaves as a single compartment with an input rate constant from the dermal cell of k_a. The expression:

$$A = \frac{M_{eluted}}{M_{cell\ t=0}} \quad (4)$$

for A is the cumulative fraction of the initial amount of solute in the outflowing perfusate at various times t is therefore given by:

$$A = 1 + \frac{k_a e^{-ke.t}}{k_e - k_a} - \frac{k_{el} e^{-ka.t}}{k_{el} - k_a} \quad (5)$$

Estimates for k_e were obtained by the nonlinear regression of A vs t using estimates of k_a from the clearance data in Table 1 and the computer program MINIM on a Macintosh Classic II. The half-life for the removal of solutes from the limb was then defined as $t_{\frac{1}{2}} = 0{\cdot}693/k_e$. The time for the peak amount of solute in the tissues was estimated from the usual equation for first-order absorption into a one compartment model:

$$t_p = \frac{1}{k_a - k_e} \cdot \log \frac{k_a}{k_e} \quad (6)$$

Iontophoresis

Hindlimb perfusions were established as above, and one glass diffusion cell positioned on the stratum corneum of the depilated perfused limb (donor cell) with a second cell fixed to the rat abdomen (receptor cell) (Singh & Roberts 1993b). Two millilitres of 50 mM HEPES buffer (pH 7·4) containing [^{14}C]lignocaine and [^{3}H]water was placed in the donor cell whilst 3 mL 20 mM isotonic HEPES buffer was placed in the receptor cell. A direct current of 0·38 mA cm^{-2} was passed between the cells via two electrodes (silver and silver chloride), with 10 μL samples taken at 15-min intervals and tissues dissected and assayed after 90-min application as described previously.

Microsphere determination of tissue flow

A known concentration ($1{\cdot}5 \times 10^5$) 15-μm ^{51}Cr-labelled microspheres (NEN-TRAC) was prepared in a 1-mL syringe as an injection mixture in 300 μL saline and 0·05% Tween 20. The actual dose of microspheres injected (D_i) (counts min^{-1}) was calculated from the difference between the radioactivity of the injection mixture and that remaining in the catheter and syringe after the injection. Microsphere studies were conducted in both the rat isolated perfused hindlimb preparation and in anaesthetized rats. In the isolated preparation, the microspheres were injected as a bolus dose into the arterial catheters of hindlimbs at perfusion flow rates (Q_p) of either 4 or 8 mL min^{-1}. Three minutes after microsphere injection, the perfusion was stopped, the hindlimb tissues completely dissected into

preweighed vials and microspheres/100 g tissue (D_t) determined in a gamma-counter (Cobra II, Packard Instruments, Australia). For flow determinations in anaesthetized rats the right carotid artery was cannulated and the catheter advanced into the left ventricle, a known dose of microspheres was injected and allowed to circulate for 3 min before the rat was killed by creating a pneumothorax. The hindlimb was dissected and the tissues removed for gamma counting. Tissue capillary flows (Q_t) in each case were determined from the following relationship (Heymann et al 1977):

$$Q_t = \frac{D_t \cdot Q_p}{D_i} \quad (7)$$

Cardiac output (CO) was used as the perfusion flow rate (Q_p) in the calculation of tissue flow rates in anaesthetized animals being the flow into which the microspheres were administered for whole body distribution. A CO value of $35{\cdot}8 \pm 3{\cdot}5$ mL $min^{-1}/100$ g body weight was used, as previously determined in the same species and size rats under identical conditions (Singh & Roberts 1993a).

Results and Discussion

Flow rate dependence of solute dermal absorption

Topically applied solutes penetrate the skin and reach underlying tissues at rates determined by a number of factors. Of principal importance is their chemical structure (Roberts 1991) and the rate at which they are removed by the local blood supply (Siddiqui et al 1989; Riviere et al 1992). The dependence of dermal clearance and subsequent tissue penetration of three solutes of differing physicochemical properties (Table 1) on tissue perfusion rate is shown in the rat perfused hindlimb. Fig. 1 shows the disappearance rates of water, diazepam and lignocaine from dermal cells at two perfusion flow rates, 4 and 8 mL min^{-1}. The dermal clearance of solutes was shown to increase with perfusion flow rate (Table 1), but not in a proportional manner. Fig. 2 shows that the extent of solute recovery in the outflowing perfusate is also a function of perfusate flow rate and solute structure. As expected, the amount of solute appearing in the outflowing perfusate was higher at the higher flow rate, corresponding to an increased clearance of solute from the dermal cell (Fig. 1, Table 1).

Table 1. Physicochemical properties of solutes used and clearances measured following dermal application to the perfused hindlimb at flow rates of 4 and 8 mL min^{-1}, mean ± s.e.m., n = 3.

	Water	Diazepam	Lignocaine
Mol. wt	18	282	234
Log P[a]	−1·15	2·82	2·37
PB (%)[b]	0	80	34
% ionized	0	1	74
pK_a	–	3·5	7·9
CL (mL min^{-1})			
at 4 mL min^{-1}	0·00135 ±0·00026	0·00155 ±0·00032	0·00092 ±0·00014
at 8 mL min^{-1}	0·00194 ±0·00020	0·00181 ±0·00009	0·00116 ±0·00013

[a]Octanol–water partition coefficient (Leo et al 1971). [b]Plasma protein binding (Wu et al, unpublished data).

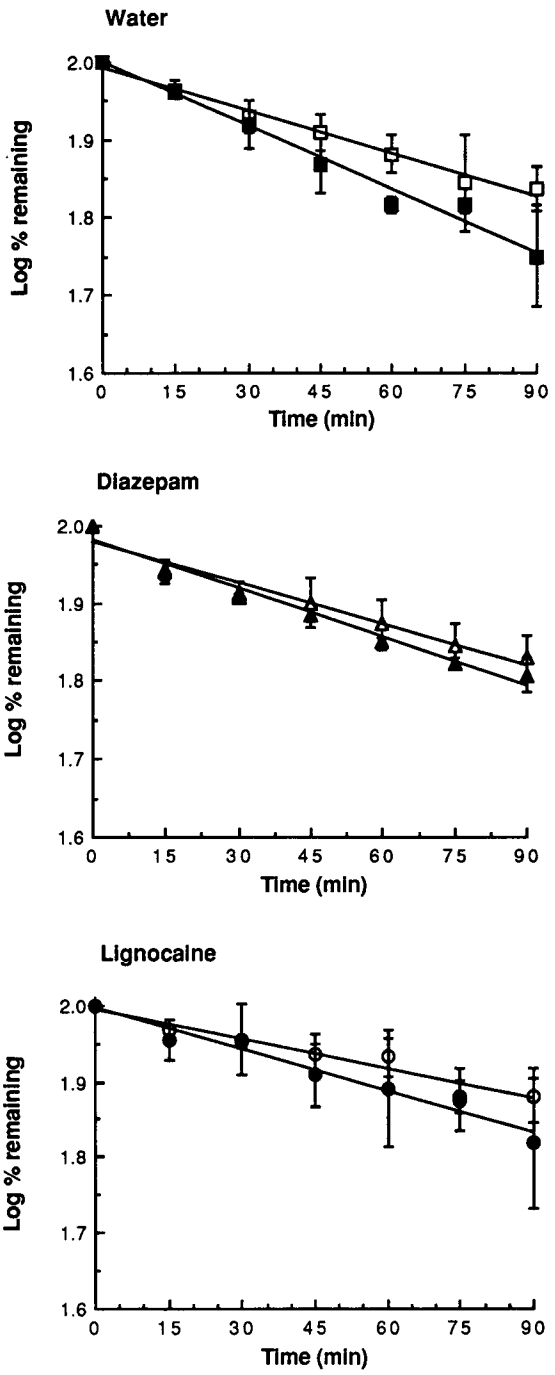

FIG. 1. Log % remaining of water, diazepam and lignocaine in dermal cells with time at perfusion flow rates of 4 (open symbols) and 8 (closed symbols) mL min^{-1} on the hindlimb. Mean ± s.d., n = 3.

Fig. 2 also shows the corresponding loss of solute from the cell and the amount estimated to be present in the tissues expressed as a fraction of the initial amount applied to the dermal cell. At the higher flow rate, the amount of solute estimated to be accumulating in the tissues appeared to reach a maximum concentration within the 90-min perfusion period. At the lower flow rate the amount of solute accumulating in the tissue appeared to increase over the 90-min period. Analysis of the outflow profiles using

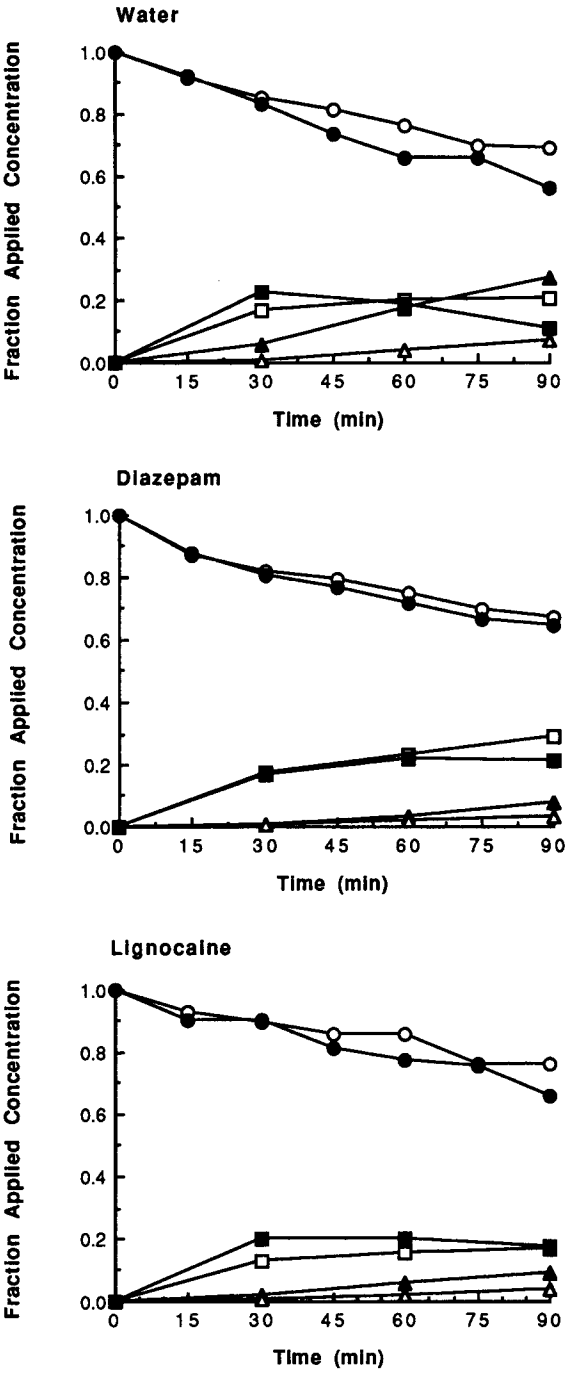

FIG. 2. Appearance of water, diazepam and lignocaine in the venous outflow with time (△), mean loss from application site (○) and estimated concentration accumulating in tissues (□) at perfusion rates of 4 (open symbols) and 8 (closed symbols) mL min^{-1}.

equation 5 confirmed the dependence of solute removal on the properties of the solute. At 4 mL min^{-1} the half-lives for removal of water, lignocaine and diazepam from the limb were 1·3, 10·1 and 36·1 min, respectively. The actual concentrations of all solutes measured in the deeper tissues at the end of the 90-min perfusion tended to be higher at the lower flow rate and dependent on the nature of the individual solute used as discussed below.

Water. The increase in clearance from the dermal cell with perfusion flow rate was most marked for water (Fig. 1, Table 1) ($P < 0{\cdot}05$, Student's t-test), where a 31% increase in clearance occurs as the perfusion flow rate is raised from 4 to 8 mL min^{-1}. This clearance perfusion flow rate dependence of water, a small un-ionized molecule, is consistent with other studies relating water dermal absorption to tissue blood flow using either the differences observed between killed and anaesthetized animals (Siddiqui et al 1989; Roberts 1991) or those in the presence of pharmacological vasoconstrictor agents (Roberts et al 1991; Cross & Roberts 1993; Singh & Roberts 1993b). The tissue accumulation of water also had the greatest dependence on perfusion flow rate as estimated by the differences in amount lost from the cell and amount eluted in the perfusate (eqn 3, Fig. 2) and the actual measured concentrations. At the higher flow rate the elution of water in the outflowing perfusate was almost 4-fold that at the lower flow (Fig. 2) and the amount of water estimated to be in the tissues at 8 mL min^{-1} was twice that at the lower flow. The decline in the estimated tissue concentration at the higher flow rate after 90 min shows that water molecules are not irreversibly sequestered in the tissue but are washed-out by the perfusion of the tissue. The estimated peak time (eqn 6) for amount in the tissues was 5·5 min.

Fig. 3A shows a plot of the actual tissue levels of water

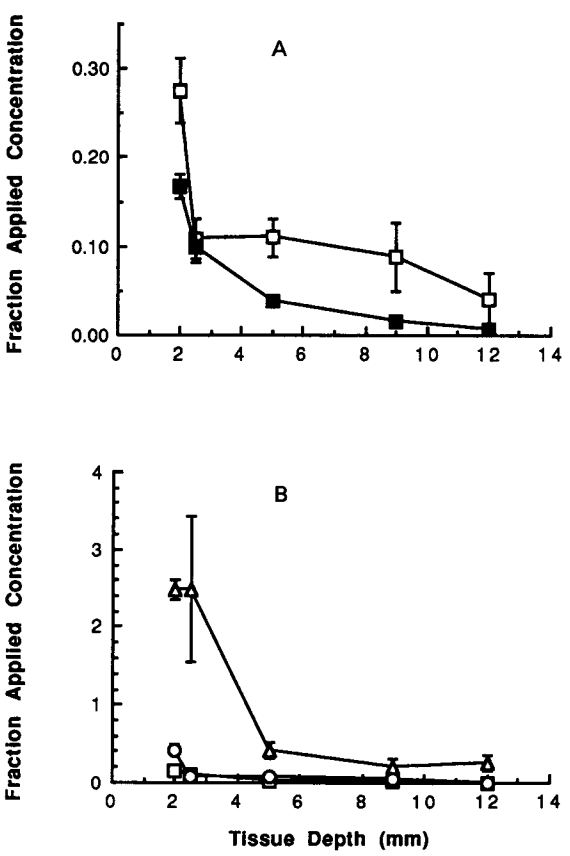

FIG. 3. A. Tissue concentrations of water following dermal application after 90-min perfusions at flow rates of 4 (open symbols) and 8 (closed symbols) mL min^{-1}. B. Tissue concentrations of water (□), diazepam (△) and lignocaine (○) following dermal application after 90-min perfusion at a flow rate of 8 mL min^{-1}. Mean ± s.d., n = 3.

measured directly beneath the application site. Tissue concentrations were statistically lower in the dermis ($P < 0.05$) and superficial muscle layer ($P < 0.05$) at the higher flow rate. These results are consistent with an increased clearance of water from the various tissues into the perfusate at the higher flow rate, as distinct from the clearance of water by diffusion into deeper tissues (Singh & Roberts 1993a). The estimated tissue concentrations of water measured at the lower flow rate are almost double those seen at the higher flow rate (Fig. 2), as predicted from the differences between the cumulative outflow and cell-loss concentrations. At the lower flow rate higher concentrations of water are found in the deeper muscle layers (Fig. 3A). This deeper penetration shows that the dermal vasculature is not acting as a near-perfect sink (Roberts et al 1991; Cross & Roberts 1993) and that certain solutes, as demonstrated clearly by the present data, can bypass this sink and diffuse into deeper tissue layers.

Diazepam. The clearance of the highly lipophilic and un-ionized solute diazepam only increased by 14.5% as the perfusion flow rate was increased from 4 to 8 mL min^{-1} (Fig. 1, Table 1). The cumulative amount of diazepam eluting in the perfusate (Fig. 2) increased 2·3-fold with the doubling in flow rate. However, an estimated maximum tissue concentration was not attained over the 90-min perfusion period for both flow rates. The peak time estimated from equation 6 was 77·0 min. These results may reflect the binding of diazepam to tissue since diazepam is highly protein bound (Table 1). The estimated amount of diazepam in the tissues at 4 mL min^{-1} is 1·37-fold that at 8 mL min^{-1}, suggesting that this amount depends both on perfusion flow rate and time to diffuse into deeper tissues. The tissue concentrations of diazepam in the dermis were approximately 10-fold the concentrations of the other two solutes, consistent with the lipophilicity and high protein-binding properties of diazepam.

Lignocaine. The clearance of lignocaine from the dermal cell decreased by 21% between flow rates of 8 and 4 mL min^{-1}. Lignocaine is a partially ionized, poorly protein-bound and fairly lipophilic solute (Table 1), and the dependence on flow rate of the estimated rate of tissue accumulation and elution of lignocaine fell between those of water and diazepam. The cumulative amount of lignocaine eluting in the perfusate was 2·6-fold that at the higher flow rate (Fig. 2). The total amount of lignocaine estimated to be accumulating in the tissues over the 90-min perfusion was not significantly different between flow rates. However, as shown with water, the decline in the estimated amount accumulating in the tissues at the 90-min time point reflects the ease with which lignocaine is being cleared from the tissues at the higher flow rate, indicating that this solute does not undergo any sequestration or irreversible binding to the tissues. The peak time estimated from equation 6 is 32·0 min. The actual tissue concentrations measured in the tissues directly below the dermal cell showed that, as exemplified by water (Fig. 3A), the tissue concentrations were slightly higher in the muscle layers at the lower flow rate.

Iontophoretic studies

Dermal iontophoresis is defined as the movement of ions through the skin when an electrical potential difference is applied (Singh & Roberts 1989). The process painlessly enhances the topical absorption of charged solutes and provides a means of obtaining higher local tissue concentrations that would otherwise be reached without high systemic administration (Glass et al 1980) or application into deeper skin layers. Given that the absorption of lignocaine applied topically to intact rat skin is minimal (Singh & Roberts 1990), the use of iontophoresis as a means of facilitating lignocaine transport through the skin of the rat perfused limb model was studied. The clearance of lignocaine (approx. 74% ionized at pH 7·4, Table 1) from diffusion cells during iontophoresis is substantial, a clearance of $7{\cdot}68 \pm 1{\cdot}6 \times 10^{-4}$ mL min^{-1} being found at a perfusion rate of 4 mL min^{-1} (Fig. 4). This value is similar to that obtained in the hindlimb after direct application of lignocaine to the exposed dermis $9{\cdot}21 \pm 1{\cdot}4 \times 10^{-4}$ mL min^{-1} at the same perfusion rate. Singh & Roberts (1994) have previously shown in the anaesthetized rat model that the rate of absorption of lignocaine using topical iontophoresis was similar to that obtained by passive diffusion after direct application to the dermis. The absorption of lignocaine was enhanced by iontophoresis to a greater extent than water (Fig. 4) as was anticipated from the difference in iontophoretic delivery rates reported for charged (lignocaine) and uncharged (water) solutes. It is suggested that neutral solutes are transported by a convective or volume flow effect (Yoshida & Roberts 1993).

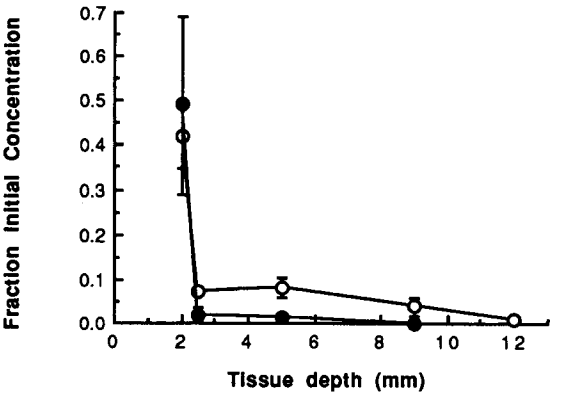

FIG. 4. Tissue concentrations of lignocaine after passive dermal application (open symbols) and topical iontophoresis (closed symbols). Mean ± s.d., n = 3.

The pattern of tissue distribution of lignocaine was similar to that observed following dermal application (Table 2). However, iontophoresis resulted in slightly lower concentrations of lignocaine in the deeper tissues

Table 2. Clearance (mL min^{-1}) of lignocaine and water after passive dermal application and topical iontophoresis.

	Lignocaine	Water
Passive dermal application	0·00092 ±0·00014	0·00135 ±0·00026
Topical iontophoresis	0·00078 ±0·00016	0·00035 ±0·00016

Mean ± s.d. (n = 3).

than seen following application to the exposed dermis. The lower concentrations may reflect a more lateral movement of the solute through the upper tissue layers towards the electrode in the receptor cell during iontophoresis, with an overall reduction in penetration relative to passive diffusion from the dermis.

Microsphere determination of tissue perfusion

The extent of tissue capillary perfusion in the hindlimb determined by microsphere injection at flow rates of 4 and 8 mL min^{-1} are shown in Fig. 5. Microspheres (15-μm diam.) are trapped as they enter the nutritive capillaries (average capillary diam. 1–8 μm) of the vascular beds they perfuse, and have therefore been extensively used to quantify regional capillary blood flow (Hales 1974; Hales et al 1979). We used this technique to determine how tissue perfusion was distributed and whether the increase in the femoral perfusion rate from 4 to 8 mL min^{-1} was reflected in the perfusion rates of tissues underlying the absorption sites on the hindlimb. In general, the tissue flows measured in the deeper muscles of the perfused hindlimb were higher than those found in the anaesthetized-rat model (Fig. 5), which appeared to show a fairly uniform distribution of flow across the tissue layers of the hindlimb. The flows are also slightly higher than those measured by Singh & Roberts (1993a) for similar depths of tissue on the anaesthetized rat abdomen. The differences probably reflect the differences in sites and a high degree of vasodilatation in the deeper muscles in the artificially perfused hindlimb model. The results from the present study demonstrate that increasing the perfusion rate through the femoral artery results in a greater recruitment of capillaries in the deeper tissues compared with the more superficial structures such as the skin and superficial muscle. A 5-fold increase in flow was seen in the deep muscle of the hindlimb (9–12 mm below the skin surface) as the perfusion rate was increased from 4 to 8 mL min^{-1}. In contrast, the same change in flow resulted in only a 2-fold increase in the flow to the skin and superficial muscle. This result implies that the flow to the superficial structures at 4 mL min^{-1} could already be at the maximum possible in the preparation. There was no significant

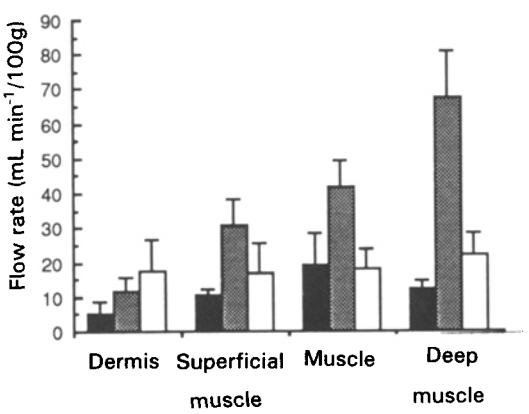

FIG. 5. Blood flows to dermis, superficial muscle, muscle and deep muscle beneath the site of dermal cell application at perfusion flow rates of 4 (■) and 8 (▒) mL min^{-1}, and in the anaesthetized rat (□), mean ± s.d., n = 3.

increase in the wet weight of the perfused hindlimbs between the perfusions at 4 and 8 mL min^{-1}, and therefore no reason to suggest oedema accounted for the increased microsphere distribution in the deeper tissues, hindlimb weights being 7·52 ± 0·42 and 7·49 ± 0·07% of total body weight, respectively. There is, therefore, a greater capacity for capillary recruitment in the deeper muscles and, it can be predicted that, as a consequence solutes reaching this depth will be cleared much more rapidly at higher flow rates than would be expected.

The outflowing perfusate collected during the 3-min period after the microsphere injection contained minimal amounts of radioactivity, demonstrating that most of the microspheres were being trapped by the microcirculation and were not bypassing capillary beds through arteriovenous anastomoses (AVAs). The existence and blood flow through AVAs has been demonstrated in various organs using the microsphere wash-out technique (Hales 1974; Hales et al 1982). Hales et al (1982) determined the extent of AVA flow in the sheep hindlimb and reported that in a warm thermoneutral environment and in the absence of drugs, 24% of the femoral blood flow passed through AVAs. In the present study, at both flow rates, less than 0·5% of the microsphere dose injected emerged in the outflowing perfusate, indicating that either only this proportion of AVAs are present in the rat hindlimb or, more likely, that due to the vasodilation present and the extent of capillary flow, none of the AVAs present are being used.

Conclusion

Good reproducibility was obtained for the dermal absorption of three solutes with differing physicochemical properties at two perfusion flow rates using the rat perfused hindlimb model. The variation of absorption rate with increasing perfusion rate was most marked for the small un-ionized water molecule, with the large un-ionized, lipophilic solute diazepam showing the least dependence. The model also showed that the rate of topical lignocaine delivery by iontophoresis through intact skin was the same as that after direct dermal application, although the subsequent deeper tissue levels obtained were lower using iontophoresis. During perfusion, the volume of capillary flow in deeper muscles increased 5-fold with a doubling of perfusion flow rate from 4 to 8 mL min^{-1}, compared with only a 2-fold increase in the skin and superficial tissues, demonstrating an extra capacity for capillary recruitment in the deeper muscles in response to increased perfusion. The data obtained on flow rates in the tissues of anaesthetized rat showed that the existing model appeared to be in a state of vasodilatation; therefore, for future studies means of maintaining vascular tone need to be investigated. The rat perfused hindlimb technique has great potential for use in a wide range of studies of the kinetics of topical solute absorption due to its capacity for the combination of many of the advantages of existing in-vitro and in-vivo models.

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