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Transdermal permeation of vasopressin. I. Influence of pH, concentration, shaving and surfactant on in vitro permeation

Partha S. Banerjee * and Wolfgang A. Ritschel

Division of Pharmaceutics and Drug Delivery Systems, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267 (U.S.A.)

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Summary

Sorption and desorption isotherms of vasopressin (VP) were determined using rat epidermis. Sorption affinity was low and desorption was rapid indicating that binding to skin is of minor importance in the skin permeation of vasopressin. Effect of pH, donor phase concentration, surfactant and prior shaving on in vitro skin permeation was investigated using excised rat skin. Percutaneous absorption of VP is not the best at or near its isoelectric point (10.9). It is higher at pH 5.0, corresponding to its higher 1-octanol/buffer partition coefficient at that pH. VP exhibited best permeation properties when the donor phase concentration was 49.5 μg/ml. The concentrations studied were 10.85 to 396.0 μg/ml. Sodium lauryl sulfate, an anionic surfactant, had no significant effect on VP skin permeation. Close shaving of the skin, 24 h before application increased flux 5 times over the control, compared to 70 times increase after stripping 25 times with cellophane tape.

Introduction

Since the introduction of the transdermal delivery system for scopolamine (Chandrasekaran, 1983) transdermal delivery of drugs for systemic action has gained considerable interest. Advancement in transdermal technology (Shaw and Urquhart, 1980) eliminated the unpredictability associated with the transdermal route in the past. The advantages of transdermal route (Shaw and Chandrasekaran, 1978) are avoidance of the variable absorption and first-pass metabolism with oral therapy, continuous drug input, permitting use of drugs with short elimination half-lives, ability to terminate drug effect rapidly and improved patient compliance. However, because of the skin's impermeability only relatively potent agents can be used by this route.

Peptides have received much attention in recent years (largely due to advancement in recombinant DNA technology to produce large amounts of these compounds) as the drugs of the future. Since they are destroyed in the gastrointestinal tract, several other potential routes have been investigated to develop an efficient drug delivery system.

* *Present address*: School of Pharmacy, University of Wisconsin, Madison, WI 53706, U.S.A.
Correspondence: W.A. Ritschel, Division of Pharmaceutics and Drug Delivery Systems, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267-0004, U.S.A.

Percutaneous route has remained least explored primarily because of the large size and ionic nature of the peptides and the low permeability of skin to such molecules. The early studies (Rennie, 1923; Telfer, 1923; MacLeod and Campbell, 1925; Harrison, 1926; Hermann, 1935; Hermann and Kassowitz, 1935) on the percutaneous absorption of insulin show conflicting results. Major (1936) used a variety of vehicles on the shaved abdomen of rabbits to enhance absorption of insulin. Bruger and Flexner (1936) concluded that recently abraded skin such as produced by shaving, permits the absorption of an appreciable amount of insulin. Standard insulin solution lowered blood sugar in rabbits (Russell et al., 1946) if the skin was pretreated with chloroform or petroleum ether. However, insulin solutions incorporated in ointment bases failed to lower the blood sugar level after application to the abdominal skin with or without pretreatment. Topical absorption of vasopressin and corticotropin in rat skin was slightly increased when applied after application of 70% DMSO at a dose approximately 20,000 times the i.v. dose (Kastin et al., 1966). The VP effect lasted only up to 40 min, which was too short to evaluate percutaneous absorption of peptides. Menasche et al. (1981) studied the percutaneous penetration of elastin peptides and found that approximately 40% of the peptides administered remain in the dermis, indicating penetration of elastin peptides through the stratum corneum. Studies in cosmetics indicate that penetration of large proteins such as collagen into the skin is controversial (Chvapil and Eckmayer, 1985) but partial hydrolysates of collagen are capable of reaching the deeper skin layers where they may serve as a source of amino acids (Schuster and Domsch, 1984). All these studies (except vasopressin) involved molecules of large size (MW > 3000), which are unlikely to go through skin (Idson, 1975). The skin steady-state permeation profiles and influence of different formulation factors on skin permeation of smaller peptides have not been studied systematically.

Vasopressin, a cyclic nonapeptide (MW 1084), is primarily used in diabetes insipidus. Recently vasopressin has been shown to have potential application in memory and learning disorders (Van Ree et al., 1985). It has a short half-life (10–20 minutes) and is highly potent. The normal plasma level desired is 0.5 to 7 pg/ml (Kutsky, 1981). It has been reported (Robertson, 1974) that in patients with pituitary diabetes insipidus the plasma vasopressin level remained low when plasma osmolality rose whereas in normal subjects it rose along with plasma osmolality. Hence vasopressin must be administered continuously to those patients. Since therapy is lifelong, the drug delivery system also must be effective and convenient. The presently prevalent i.m. or s.c. administration has longer duration of action but erratic absorption and inconvenience of parenteral administration make it less than ideal for long-term therapy. In this perspective, the transdermal route appears to be the ideal one for vasopressin.

The present study was undertaken to investigate the skin binding and effect of pH, concentration and surfactant on the in vitro skin permeation of vasopressin. The effect of pH on skin permeation was correlated with the 1-octanol/buffer partition coefficient. Also, the effect of shaving on the skin permeation behavior was studied. The drug chosen (vasopressin) also represents a model for peptides of similar molecular size.

Materials and Methods

Materials

Vasopressin was purchased from Sigma. Tritium-labeled vasopressin (vasopressin, 8-L-arginine, [phenylalanyl-3,4,5-^{3}H(N)]) (spec. act. 64–70 Ci/mmol) was supplied by New England Nuclear. The radiochemical purity of labeled vasopression was checked by thin layer chromatography on Silica gel G plates (Eastman Kodak) using a *n*-butanol : acetic acid : water : pyridine (15 : 10 : 12 : 1) solvent system. It exhibited a single peak containing more than 98% of the radioactivity (detected by scintillation counting), with a R_f value of 0.68. Sodium lauryl sulfate was obtained from Sargent. Scintillation Cocktail 3a70B (Research Products International) was used for scintillation counting. Solvents and

buffer substances used were of reagent grade. All solutions were made with deionized water.

Skin

Female Sprague–Dawley rats (Harlan) of 10–20 weeks of age were sacrificed by overdose ether inhalation. The skins, excised after light clipping of the hair on the back with electric clippers (Oster), were cleaned of subcutaneous tissue and fat. The skin was mounted on diffusion cells and used within an hour as described below.

Binding study

Full-thickness excised rat skin was used. Epidermis was separated from dermis by a previously reported method (Scott et al., 1986). The sorption isotherm was determined by equilibration of a measured weight of epidermis with 1 ml known concentration of radiolabelled vasopressin solution in a shaker-bath for 3 h at 30 °C. Concurrent studies indicated that equilibration was achieved within 1 h. After equilibration, the epidermis was digested with TS-1 tissue solubilizer (Research Products International) and analyzed by scintillation counting. Duplicate samples (10 μl) were also taken from the aqueous phase and analyzed.

For desorption study, epidermis pieces, after equilibration with radiolabelled vasopressin solution, were blotted dry with a tissue paper and placed into a vial containing 1 ml deionized water. Samples (20 μl) were taken with replacement with deionized water at selected time intervals and analyzed by scintillation counting.

In vitro skin permeation

Full-thickness excised rat skin was mounted on a single chamber Franz glass diffusion cell (Vangard International) across a 2 cm diameter opening at the top of the cell with the stratum corneum facing upwards. The receiver chamber had a volume of 8 ml and was filled with pH 7.4 isotonic phosphate-buffered saline (ionic strength: 0.26 M). It was kept at 37 °C by circulating water through an external jacket. After 1 h equilibration of the skin with receptor fluid, 200 μl of the drug solution was applied uniformly on the stratum corneum side. The donor phase was covered with Parafilm to prevent evaporation of the solvent. Receptor phase samples (100 μl) were taken at selected time intervals with replacement with phosphate-buffered saline and analyzed by liquid scintillation counting.

The effect of pH was studied at pH 5.0, 7.4 and 10.9. The pH values were selected to reflect the skin surface pH (5.0) and body fluid or receptor fluid pH (7.4) (two extreme pH's likely to be encountered by the molecule during permeation) and also the isoelectric point of vasopressin (10.9) where it is electrically neutral. Prior to the pH study, the stability of vasopressin at pH 5.0 and 10.9 were studied at 30 °C for 2 days and compared with freshly prepared solution by TLC. Both the freshly prepared and sample solutions had a single peak with the same R_f values. Skin metabolism under the conditions of the in vitro experiment was also determined by incubating full-thickness excised rat skin treated with radiolabelled vasopressin solution in pH 7.4 isotonic phosphate buffered saline for 2 days at 37 °C. A control was run without any skin. After incubation, the solutions were analyzed for the appearance of any degradation product by thin-layer chromatography. Control and sample both showed a single peak with the same R_f values. The effect of pH 10.9 buffer on the integrity of skin samples was checked by studying the permeability of tritiated water (ICN) in pH 10.9 buffer and comparing it to the control (tritiated water alone). Sodium acetate/acetic acid buffer (pH 5.0), phosphate buffer (pH 7.4) and glycine/NaOH buffer (pH 10.9) solutions, all of equal ionic strength (0.1 M) were prepared in water. Vasopressin solutions of 49.5 μg/ml were made in each buffer and were spiked with radiolabelled vasopressin (7 μCi/ml).

Six concentrations of vasopressin (10.85 μg/ml, 21.70 μg/ml, 49.5 μg/ml, 99 μg/ml, 198 μg/ml and 396 μg/ml) were used to study the effect of donor phase concentration on skin permeation. Each solution was spiked with 4–5 μCi/ml of tritiated vasopressin.

The anionic surfactant sodium lauryl sulfate was used to study the effect of surfactant. Five concentrations of sodium lauryl sulfate, 0.92, 1.92, 4.62, 7.98 and 19.2×10^{-3} M containing vasopressin (105.44 μg/ml, 4 μCi/ml) were studied.

The effect of shaving was studied by shaving rat skin one day before the study with a Remington Super Mesh Electric Shaver (Model SM-100, Remington Products Inc.) after preliminary clipping of the hair. The skin was allowed to heal for 24 h (although no visible damage was observed), and excised. This was compared to stripping, where the skin was stripped 25 times with cellophane tape (Scotch Brand, 3M) to remove stratum corneum (Flynn et al., 1981). Concentrations of vasopressin solution in the donor phase were 46.8 μg/ml (4 μCi/ml) for clipped and shaved skin and 49.5 μg/ml (4 μCi/ml) for stripped skin.

Partition coefficient determination

1-Octanol and the buffer solution in a 50 ml Teflon-lined screw cap tube were shaken for 24 h at 30 °C for cosaturation of the phases. The two phases were separated by centrifugation (model EXD, International Equipment) for 5 minutes at 800 *g*. The aqueous phase was used to prepare a vasopressin solution containing both labelled (0.5 μCi/ml) and unlabelled vasopressin (36 μg/ml). One ml of this solution was added to 1 ml of organic phase in a 7 ml screw-capped glass vial. The vials were agitated in a shaker-bath at 30 °C until equilibration. After equilibration, the two phases were separated by centrifugation and 0.5 ml of organic phase and 10 μl of aqueous phase were analyzed by scintillation counting. The apparent partition coefficient (APC) was calculated using the following equation:

$$APC = \frac{\text{concentration in octanol}}{\text{concentration in buffer}} \qquad (1)$$

Computations and statistics

The specific activity of each solution (cpm/ng of vasopressin) was determined from the radioactivity of each solution and the proportion of vasopressin. The radioactivity of samples from the receptor phase was converted from cpm/ml to ng using the receptor chamber volume and the specific activity of the solution. The permeation of vasopressin through skin was described by a plot of ng permeated vs time in h. The slope of the curve and the intercept on the *x*-axis (lag time) were determined by linear regression. Steady-state flux (ng/cm^2 · h) of vasopressin was calculated by dividing the slope (ng/h) by the area of the skin surface (3.14 cm^2) through which diffusion was taking place.

The dorsal skin of each rat was divided into 4 quadrants for the in vitro study. The drug solution at each pH, concentration and surfactant concentration was applied randomly on the quadrants. To account for the variability in rat skin permeability, the effect of pH and concentration were studied using a randomized block design with one rat as the block. Comparisons were made with Student's *t*-test or Neumann-Keuls multiple comparison range test. A probability value of less than 0.05 was considered significant.

Results and Discussion

Adsorption and desorption of the permeant by the membrane on skin transport has been reported (Wurster et al., 1979; Chandrasekaran et al., 1980). Molecules bound to skin macromolecules or surfaces are not free to diffuse. If the donor concentration is not significantly reduced by binding, steady state flux values are not affected, since flux is proportional to the concentration gradient. However, binding hinders the initial permeation of molecules through skin and the lag times are extended. The non-linear sorption isotherm can be explained by a dual sorption model (Chandrasekaran et al., 1980). This has been used to develop techniques for controlling these sorption transport processes in order to rapidly achieve predictable transdermal drug delivery in vivo.

The equilibrium epidermis concentration values of vasopressin are plotted as a function of aqueous concentration in Fig. 1. The data are also represented in the form of Langmuir adsorption isotherm (Fig. 2). The Langmuir isotherm is mathematically expressed by the following equation (Martin et al., 1983).

$$C/x/m = \frac{1}{bY_m} + \frac{C}{Y_m} \qquad (2)$$

where C is the equilibrium concentration of the

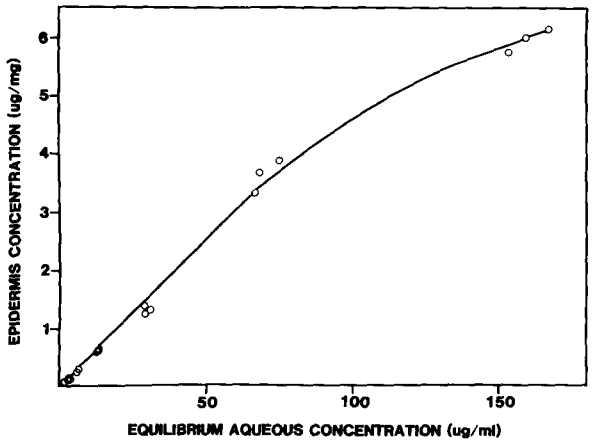

Fig. 1. Sorption isotherm for vasopressin with rat epidermis at 30 °C.

solute, x is the amount of solute adsorbed per amount m of adsorbent, b represents affinity and Y_m is the maximum adsorption capacity. The plot of $C/x/m$ vs C should yield a straight line, and Y_m and b can be obtained from the slope and intercept. Fig. 2 gives a Y_m value of 71.43 μg/mg and b value of 6.44×10^{-4} ml/μg. The very low value of b suggests a low affinity of vasopressin for the binding sites in the epidermis.

When desorption is first-order and with the assumptions that upon desorption, the adsorbate does not return to the adsorbent surface and the volume of desorbing solvent remains constant, it has been shown that (Patrick and Payne, 1961)

$$\log \frac{X_e}{X_e - X} = \frac{k_d t}{2 \cdot 303} \qquad (3)$$

where X_e is the equilibrium amount of adsorbate

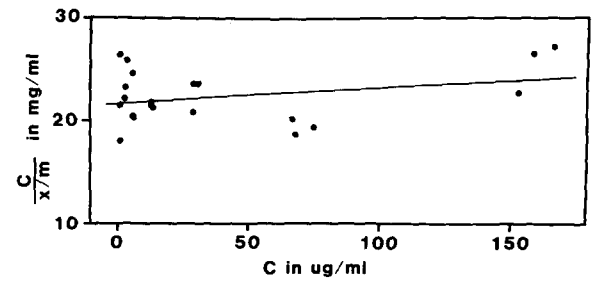

Fig. 2. Langmuir plot of the sorption isotherm of vasopressin with rat epidermis at 30 °C.

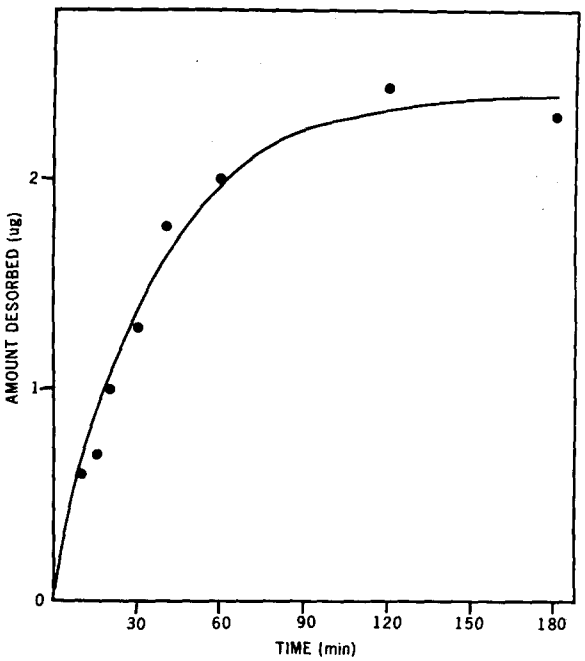

Fig. 3. Desorption isotherm for vasopressin from rat epidermis at 30 °C.

in solution and X is the amount in solution at time t. The desorption isotherm of vasopressin at 30 °C is shown in Fig. 3 for one of the 3 replicates. The first-order plot of desorption data using Eqn. 3 yielded a mean desorption rate constant of 0.0466 ± 0.0125 (S.D.) min^{-1}. The rapid desorption is probably a consequence of the low affinity of vasopressin for binding sites in epidermis as found in the sorption study. This is in contrast to large protein molecules which are found either not to desorb or to desorb only with great difficulty from quiescent interfaces (Macritchie, 1978). The sorption and desorption study indicates that binding to skin is not likely to have any significant impact on the skin permeation of vasopressin.

Estimates of steady state flux and lag time obtained after linear regression of skin permeation data at different pH are shown in Table 1. Also shown are the apparent 1-octanol/buffer partition coefficients. A graphic presentation of the inter-relationship of flux and partition coefficients and their dependence on pH is made in Fig. 4. Differences in pH can have a major impact on skin permeation of ionizable compounds (Menczel

TABLE 1

Steady state flux, lag time and octanol/buffer partition coefficient of vasopressin at different pH

	pH = 5.0	pH = 7.4	pH = 10.9
Flux (ng/cm^2·h)	3.56 ± 0.90	1.25 ± 0.92	1.63 ± 0.90
Lag time (h)	7.67 ± 2.64	2.16 ± 6.67 *	1.67 ± 2.64 *
Octanol/buffer partition coefficient × 10^3	2.26 ± 0.38	1.54 ± 0.32	0.962 ± 0.24

Values are mean ± S.D.; $n = 3$ at pH 7.4 and $n = 4$ at pH 5.0 and 10.9. Values underscored by the same line are not significantly different by Neumann-Keuls test for comparison.
* Not significantly different from zero.

and Goldberg, 1978), the permeation of unionized species being much higher than ionized species (Swarbrick et al., 1984). Vasopressin has a double positive charge at pH 5.0 and is neutral at pH 10.9, its isoelectric point. According to the pH–partition hypothesis when drugs permeate predominantly lipophilic biologic membranes by passive diffusion, the unionized species permeates more compared to the ionized species because of its higher membrane partitioning. Hence, the steady state flux of vasopressin should increase with pH, becoming highest at pH 10.9. On the contrary the flux was significantly higher at pH 5.0 than at pH 10.9. The 1-octanol/buffer partition coefficient also decreased with increasing pH and was significantly different at each pH. A

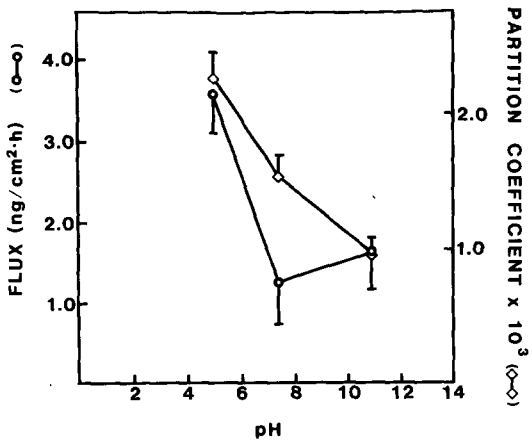

Fig. 4. Effect of pH on steady state transdermal flux and octanol/buffer apparent partition coefficient of vasopressin.

possible explanation could be that VP is forming aggregates at pH values close to its isoelectric point (neutral). This suggests that the percutaneous absorption of vasopressin may not be the best at or near its isoelectric point. This may be the same for the membrane permeation of other peptides. Rectal absorption of insulin (Ichikawa et al., 1980) was lowest at its isoelectric point corresponding to its minimum solubility (Ritschel and Ritschel, 1984). Self-association of peptides is paralleled by a decrease in their solubility (Tonido et al., 1985). This seems to be a plausible explanation for the decrease in the partition coefficient and flux in this study with increasing pH. Higher flux at pH 5.0 can be also explained by the permselective properties of skin (Burnette and Ongpipattanakul, 1987). Since the skin has a net negative charge at the physiological pH, positively charged vasopressin molecules may permeate more favorably. However this does not explain the flux at pH 7.4 and 10.9. Although the partition coefficient was significantly lower at pH 10.9 than at pH 7.4, flux was not different. At a pH higher than 9, significant reduction in skin impedance has been reported (Allenby et al., 1969). The integrity of the skin was checked by tritiated water permeability. No difference was observed with control (pH 7). The control flux values of 0.511%/cm^2 · h and 0.579%/cm^2 · h at pH 10.9 for water were comparable to 0.656 reported in the literature (Bronaugh and Stewart, 1985).

The flux, lag time and permeability coefficient at different donor phase concentrations of vasopressin are shown in Table 2. The steady state flux (J_s) can be related to the Stratum corneum/vehicle partition coefficient (K_m), diffusion coefficient in skin (D_m), concentration difference between the donor phase (C_s) and receptor phase (C_r) and the thickness of the barrier (h_m) by the following equation:

$$J_s = \frac{K_m \cdot D_m}{h_m} \cdot (C_s - C_r) \qquad (4)$$

Under sink condition in the receptor phase ($C_s \gg C_r$), C_s approximates the concentration gradient. Hence, steady state flux should increase linearly with donor phase concentration. The permeability

TABLE 2

Permeation of vasopressin through rat skin in vitro from different donor phase concentrations

Vasopressin (μg/ml)	Flux (ng/cm^2·h)	Lag time (h)	Permeability coefficient $\times 10^5$ (cm/h)
10.85	0.169 ± 0.0326	−0.0428 ± 3.04	1.56 ± 0.30
21.70	1.05 ± 0.525	6.22 ± 0.83	4.84 ± 2.42
49.50	3.46 ± 2.29	5.88 ± 4.17	7.00 ± 4.62
99.00	3.50 ± 1.05	0.035 ± 4.61	2.52 ± 1.06
198.0	4.63 ± 2.78	3.33 ± 8.03	2.34 ± 1.41
396.0	7.70 ± 2.25	0.183 ± 7.47	1.94 ± 0.569

Values are mean ± S.D., $n = 3$–4.

TABLE 3

Steady state flux and lag time of vasopressin in presence of different concentrations of sodium lauryl sulfate (SLS)

SLS concn. (M) $\times 10^3$	Flux (ng/cm^2·h)	Lag time (h)
0.00	3.27 ± 3.25	7.36 ± 4.31
0.92	4.42 ± 2.76	8.46 ± 7.97
1.92	1.37 ± 0.225	6.17 ± 1.30
4.62	1.10 ± 0.45	−4.28 ± 14.88
7.98	0.555 ± 0.338	3.71 ± 2.85
19.20	0.774 ± 0.093	2.24 ± 2.92

Values are mean ± S.D., $n = 3$.

coefficient obtained by dividing J_s by C_s should remain unchanged with increasing concentration. This study where all the concentrations were well below its saturation point, however, shows that vasopressin flux is not linearly related to donor phase concentration although it increased gradually with increased concentration. Permeability coefficient was also not unchanged. It increased with concentration, was highest at 49.50 μg/ml and lower and essentially constant at higher concentrations. At higher concentrations (99 μg/ml to 396 μg/ml) and also at 10.85 μg/ml, the permeation profile was quite variable as seen in the high variability in lag time and negative lag time obtained in some instances. The negative lag time in membrane permeation studies can be explained by simultaneous self-association and diffusion of the drug (Dressman et al., 1982), however this does not explain the behavior at lower concentration. The mean lag time at these concentrations were not significantly different from zero. From this study it appears that vasopressin has best permeation properties at or around 50 μg/ml.

The steady state flux and lag time data of vasopressin in presence of different concentrations of sodium lauryl sulfate are shown in Table 3 and Fig. 5. It has been reported (Scheuplein and Ross, 1970) that dilute solutions of ionic surfactants may alter the physical state of the skin reversibly and thus promote passage of charged hydrophilic substances. The mechanism appears to be related to the tendency of these molecules to bind to epidermal proteins and disrupt their long range order (Breuer, 1979). Among anionic surfactants, the laurate ion permeates best and has the greatest effect on the permeation of other solutes (Barry, 1983). It was speculated that the surfactant may enhance permeation either by disrupting skin structure or by forming a complex with positively charged vasopressin molecules and thus escaping the skin's charge barrier. The results, however, show that sodium lauryl sulfate had no appreciable effect on the transdermal flux of vasopressin. Lower flux observed at higher concentrations of the surfactant is most likely due to micellar trapping of the drug.

Early studies on insulin noted (Bruger and Flexner, 1936) that shaving of the skin prior to application did permit the absorption of an appreciable amount of insulin. For many peptide hormones used in acute conditions, this can be a

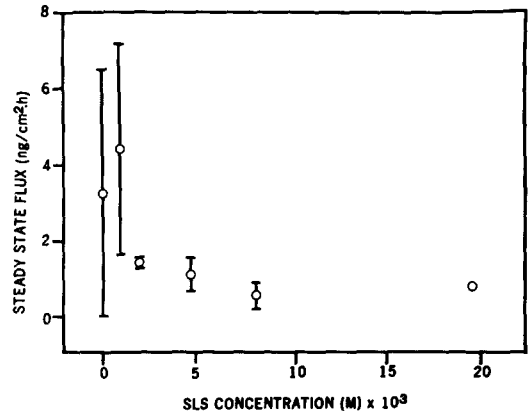

Fig. 5. Influence of the concentration of sodium lauryl sulfate (SLS) on the steady state flux of vasopressin.

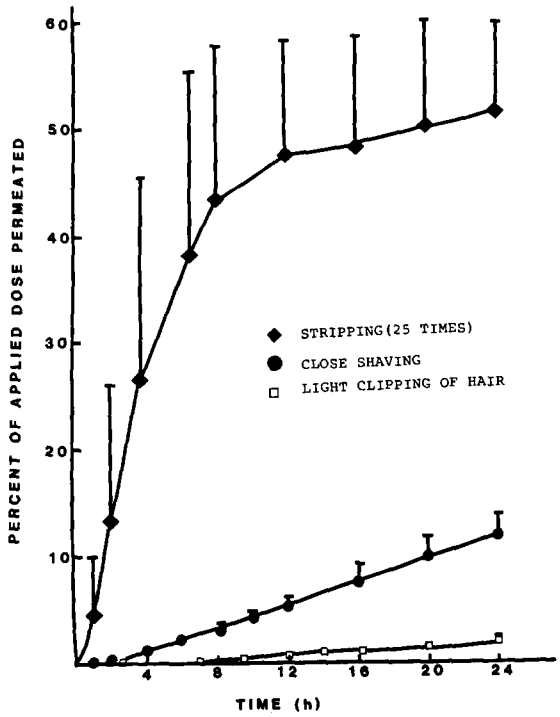

Fig. 6. In vitro skin permeation–time profile of vasopressin (mean ± S.D.) at different conditions of skin.

useful approach, provided the patient complies with it. The skin permeation–time profile after shaving and stripping is shown in Fig. 6. Flux and lag time estimates are presented in Table 4. Shaving increased the flux (about 5 times) and decreased the lag time of vasopressin significantly when compared to light clipping. Stripping (25 times), which removes the stratum corneum showed dramatic increase in VP skin permeation. This shows that shaving damages the skin partially and gives evidence to the fact that the stratum corneum is the main barrier to permeation of vasopressin.

TABLE 4

Steady state flux and lag time of vasopressin at different conditions of skin

Skin condition	Flux ($\%/cm^2 \cdot h$)	Lag time (h)
Light clipping of hair	0.0354 ± 0.0102	5.646 ± 1.058
Close shaving	0.177 ± 0.0346	2.38 ± 1.46
Stripping (25 times)	2.30 ± 1.48	0.603 ± 0.545

Values are mean ± S.D., $n = 4$.

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