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Held in Dayton, Ohio on October 30, 31 and November 1, 1984.

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IN VITRO METHODOLOGY FOR PERCUTANEOUS ABSORPTION STUDIES

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INTRODUCTION

The absorption through the skin of toxic compounds can be significant and must be considered when dermal contact occurs. In vitro methods for measuring percutaneous absorption have been demonstrated to give values in good agreement with in vivo results in human (Franz, 1975; Franz, 1978) and animal (Bronaugh, et al., 1982) studies. Rates of absorption can be measured more accurately by in vitro techniques since sampling takes place directly below the barrier layer. Procedures are discussed for performing these measurements in the static and flow-through diffusion cell. A method for the determination of the absorption of hydrophobic compounds using in vitro techniques is described (Bronaugh and Stewart, 1984).

METHODS

Radiolabeled compounds were utilized to facilitate analysis of material permeating the skin. The ^3H and ^{14}C materials were obtained from commercial sources with a radiochemical purity of at least 97%. Radioactivity was quantitated with a Beckman LS-9000 scintillation counter.

In vitro permeation studies were performed using either a static diffusion cell (Bronaugh et al., 1981) or flow-through cell system (Bronaugh and Stewart, 1985). In most experiments, rat skin was prepared by removing a 350 μm section from the surface of the skin. A circle from this section was fixed in either type of cell with the stratum corneum side up. With the static cells (area = 1.13 cm^2), aliquots were removed from the receptor at appropriate intervals for rate of absorption determinations. Samples from the flow-through cell (area = 0.32 cm^2) were automatically collected in a fraction collector. The skin surface

temperature was maintained at 32°C by placing the static cells in a 32°C water bath or placing the flow cells in a holding block heated with water at 35°C.

In vivo absorption studies in rats were performed as previously described (Bronaugh et al., 1982). Rats were partially restrained with rubber tubing and a protective device was attached to an area of lightly clipped back skin with a cyanoacrylate adhesive. The amount of material applied to the back that penetrated the skin was determined by collecting 24 hr urine samples for 5 days at which time excretion had reached background levels. These values were corrected to give total absorption by using a parenteral correction factor. This factor was determined in a separate experiment by administering the test compound to rats intraperitoneally and determining the fraction of the dose that was excreted in the urine during the same 5 day time period.

RESULTS

The amount of absorption of three compounds in the flow-through cell was compared to values obtained in the standard static diffusion cell. Similar absorption profiles were achieved with either cell for tritiated water (water vehicle, Figure 1) and for cortisone and benzoic acid (acetone vehicle, Figure 2). Almost identical steady-state absorption rates were obtained for tritiated water (Table 1). The percentage of the applied dose absorbed for benzoic acid and cortisone did not differ significantly in the two cell types (Table 1).

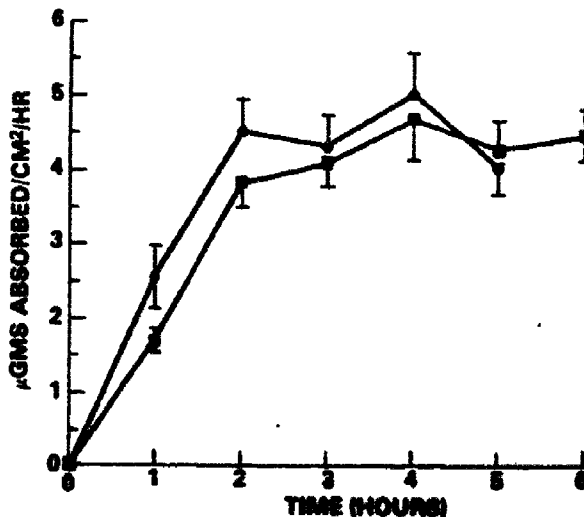


Figure 1. Comparison of tritiated water absorption in the flow-through (■) and static cells (●).

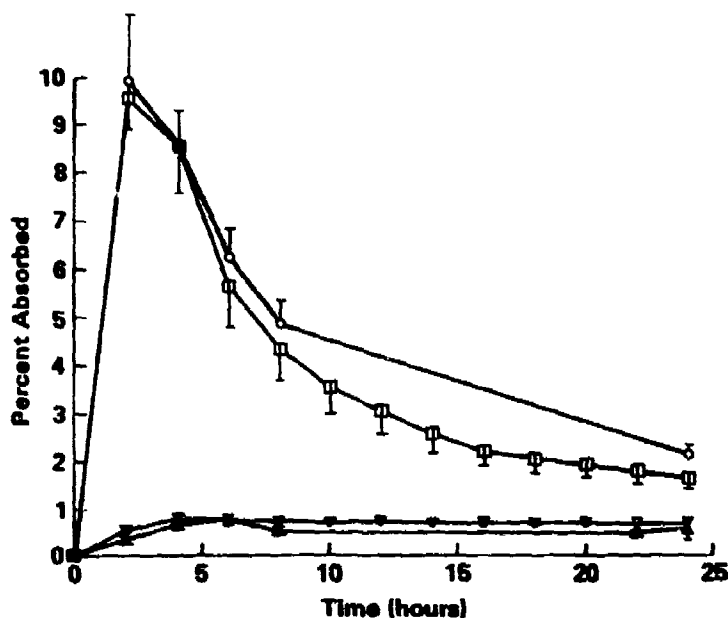


Figure 2. Comparison of cortisone and benzoic acid absorption in the flow-through and static cells. Compounds were applied to the rat skin membrane in an acetone vehicle. Flow-through cell: ▽ = cortisone, □ = benzoic acid; static cell: ▲ = cortisone, ● = benzoic acid.

TABLE 1. COMPARISON OF FLOW-THROUGH AND STATIC DIFFUSION CELLS^a

Compound	Absorption ^b	
	Flow-Through	Static
Water	4.3 ± 0.4 (5)	4.4 ± 0.2 (5)
Cortisone	8.5 ± 0.9 (5)	6.3 ± 0.8 (8)
Benzoic Acid	45.9 ± 7.6 (5)	48.6 ± 3.8 (6)

^a Values are the mean ± S.E. of the number of determinations in parentheses. [³H]Cortisone and [¹⁴C]benzoic acid were applied in an acetone vehicle, [³H]water in a water vehicle. The values obtained for each compound in the two types of cells were not significantly different from each other when compared by the two-tailed Student's t-test, $p < 0.05$.

^b For cortisone and benzoic acid, absorption is expressed as the percentage of the applied dose absorbed in 24 hr. For water, the steady-state rate of absorption of the radiolabeled molecule is given ($\mu\text{g}/\text{cm}^2\text{hr}$).

Permeation data from the diffusion cells were compared with values for the absorption of benzoic acid and cortisone in living rats. Petrolatum was used as the vehicle in this series of experiments. In both the in vivo and in vitro studies, the site of application was washed after 24 hr. Good agreement was seen in the permeation data from the three procedures (Table 2).

TABLE 2. COMPARISON OF IN VIVO AND IN VITRO ABSORPTION (PERCENTAGE APPLIED DOSE ABSORBED)^a

Compound	In Vivo	In Vitro	
		Flow Cell	Static Cell
Cortisone	19.6 ± 1.3 (4)	20.1 ± 1.1 (6)	22.8 ± 2.7 (5)
Benzoic Acid	37.0 ± 2.8 (8)	28.3 ± 3.0 (6)	35.5 ± 5.2 (5)

^a Values are the mean ± S.E. of the number of determinations in parentheses. Compounds were applied in a petrolatum vehicle. The values obtained for each compound by the three methods were not significantly different from each other when compared by the two-tailed Student's t-test, $p < 0.05$.

For the hydrophobic fragrance ingredient, cinnamyl anthranilate (CA), in vitro percutaneous absorption measurements resulted in values that were much lower than the in vivo data (Table 3). The effect of different receptor fluids on CA absorption was determined by comparing values obtained after 5 days. Only 12.7% of CA absorbed in vivo during the 5 day period was obtained in a corresponding in vitro experiment, using saline receptor fluid and dermatomed rat skin. A 1.5% solution of the nonionic surfactant oleth 20 enhanced by three-fold the skin permeability to CA; the absorption of the cortisone control, determined simultaneously in a dual-label experiment, was not altered. The optimal concentration of oleth 20 was 6%. A five-fold increase in the absorption of CA was obtained without altering the permeation of the cortisone control.

Rabbit serum and bovine serum albumin had no effect on the integrity of the barrier, but they were also less effective in increasing CA absorption than oleth 20. A methanol-water solution and 6% octoxynol 9 were equal or superior to 6% oleth 20, but significant damage to the skin was indicated by the increased cortisone permeation since the absorption of cortisone is not

limited by a lack of solubility in the normal saline receptor fluid. A 6% solution of poloxamer 188 in the receptor resulted in slight enhancement of both CA and cortisone permeation.

TABLE 3. EFFECT OF DIFFUSION CELL CONDITIONS ON THE ABSORPTION OF CINNAMYL ANTHRANILATE (CA) (CORTISONE CONTROL)^a

<u>Receptor Fluid</u>	<u>CA, % Applied Dose Absorbed (5 days)</u>	<u>Cortisone Permeability Constant X 10⁵</u>
Normal saline (4) ^b	5.0 ± 0.3	3.8 ± 0.7
1.5% oleth 20 (4) ^b	5.4 ± 0.9	-
Normal saline (4)	5.8 ± 0.4	7.1 ± 0.5
1.5% oleth 20 (10)	15.5 ± 1.2 ^c	6.1 ± 0.5
6% oleth 20 (8)	27.9 ± 1.8 ^c	7.0 ± 0.9
20% oleth 20 (8)	18.3 ± 1.8 ^c	9.3 ± 0.9
Rabbit serum (4)	8.8 ± 0.6 ^c	6.8 ± 0.8
3% bovine serum albumin (4)	12.1 ± 1.2 ^c	5.4 ± 0.2
50:50 methanol-water (4)	27.1 ± 2.0 ^c	17.2 ± 0.2 ^c
1.5% octoxynol 9 (4)	17.9 ± 1.1 ^c	10.8 ± 0.5 ^c
6% octoxynol 9 (4)	38.4 ± 2.9 ^c	14.5 ± 1.3 ^c
6% poloxamer 188 (4)	7.3 ± 1.3	9.8 ± 0.6c

^a Values are the mean ± S.E. of the number of determinations in parentheses. For most experiments, a 350- μ m section from the surface of whole rat skin was prepared with a dermatome. Compounds were applied to the skin in a petrolatum vehicle. In vivo absorption of I was 45.6%.

^b Whole skin.

^c Significant increase when compared to results from saline (dermatome section) by one-tailed Student's t-test, $p < 0.05$.

The effect of 6% oleth 20 on the absorption of water and urea was examined with rat skin in diffusion cells (Table 4). No significant increase was seen in the percutaneous absorption of these two additional control compounds.

TABLE 4. EFFECT OF OLETH 20 ON THE ABSORPTION OF WATER AND UREA^a

<u>Receptor Fluid</u>	<u>Water Permeability Constant X 10³</u>	<u>% Absorbed (43 hr)</u>	
		<u>Water</u>	<u>Urea</u>
Normal saline	2.5 ± 0.6	21.0 ± 5.4	9.3 ± 3.2
6% oleth 20	2.7 ± 0.6 ^b	23.5 ± 5.5 ^b	10.3 ± 3.4 ^b

^a Values are the mean ± S.E. of eight determinations. Compounds were applied to rat skin (350- μ m section) in 600 μ L of water.

^b No significant increase when compared with saline controls by one-tailed Student's t-test $p < 0.05$.

DISCUSSION

Good correlation has been found between the static and flow-through cell procedures. The flow-through cell has the advantages of automatic sampling (with a fraction collector) and sample collection 24 hr daily. With regard to accuracy, neither cell is superior and both were found to give absorption values similar to the in vivo data.

The nonionic surfactant (oleth 20) was found to be beneficial in the determination of the in vitro absorption of hydrophobic compounds. The permeation of the fragrance cinnamyl anthranilate (water solubility = 0.23 mg/liter) was markedly enhanced without apparent damage to the skin.

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