THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 67:713–717, 1976

Vol. 67, No. 6
Printed in U.S.A.

THE IN VITRO PERMEABILITY OF SKIN AND BUCCAL MUCOSA TO SELECTED DRUGS AND TRITIATED WATER

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The permeability of whole human skin, human dermis, whole pig skin, and canine buccal mucosa have been determined for four chemically different solutes: tritiated water, amphetamine, estradiol, and ouabain. Several new in vitro techniques for isolation, preservation, and permeability determination of these membranous tissues are described. Water permeabilities of human epidermis and dermis using these techniques agree well with published results. Human and porcine skins are very similar with respect to water permeability, while buccal mucosa is similar to dermis in its permeability characteristics to the four representative test agents. The permeability of hydrated whole skin is determined by the permeability of the epidermis, and the dermis and buccal mucosa behave as if they are water barriers exhibiting a permeability of about 30% of the diffusion through pure water, a difference that can be ascribed to the porosity and/or tortuosity of the tissue matrix.

The permeability of human skin to various agents has been studied for a number of years [1–4]. The impetus for many of these studies was the interest in the penetration of industrial and biologic warfare toxins as well as that of therapeutic agents into diseased skin. Recently, the interest in skin and oral mucosal permeability has been renewed by the potential usefulness of skin and buccal tissues as routes of drug administration for systemic therapy [5–7]. Two factors encouraging this mode of drug delivery are the accessibility of these tissues and the potential for better control of the rate and quantity of drug delivered to the body.

This interest in topical delivery of systemic drugs has led us to investigate the permeability properties of whole skin, dermis, and buccal mucosa. It has not been our aim to exhaustively describe the permeabilities of the various tissues to every potential therapeutic agent, but to elucidate general characteristics of these tissues which will assist efforts to select appropriate drugs for topical delivery. Furthermore, we have tried to develop rapid, reliable, and accurate methods for determining tissue permeabilities in vitro and also to investigate the usefulness of pig skin as a model for human skin in permeability studies.

Manuscript received February 9, 1976; accepted for publication April 20, 1976.

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Abbreviations:
D: diffusion coefficient
$M^{1/2}$: square root of molecular weight
P_s: permeability coefficient
THO: tritiated water

MATERIALS AND METHODS

Human skin was obtained from the medial thigh of cadavers no more than 24 hr post mortem using a Padgett Model B electric dermatome (Padgett Dermatome Division, Kansas City Assemblage Co., Kansas City, Mo. 64108). The skin was ~750 μ in thickness and of a quantity sufficient for several permeation experiments.

Studies in which the fresh skin was used were conducted immediately after hydration of the skin for 12 hr in a pH 7 phosphate-buffered Ringer's solution containing 100 U/ml of penicillin and 1.0 mg/ml of streptomycin sulfate at room temperature (~22°C). It was determined that these agents had no measureable effect on membrane permeability but they prevented bacterial growth during skin hydration and subsequent experimental determinations.

Skin samples not used immediately were stored at 4°C in a sterile skin bank fluid composed of 800 ml of a balanced salt solution (consisting of 136 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 1.7 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.3 mM Na_2HPO_4, 5 mM dextrose, 3.4 mg/L phenol red, 0.5 mg/L neomycin sulfate) and 200 ml of human plasma.

Human dermal samples were obtained as described by Scheuplein [8], by immersing the whole-skin samples in 60°C water for approximately 45 sec, after which the epidermis could easily be peeled away leaving the dermis. Since the epidermis is such a small proportion of the total thickness, the thickness of the dermis thus obtained is essentially the same as that of whole skin.

Pig skin samples were taken from the backs of approximately 8-week-old weanling pigs in a manner similar to that described above for human skin. This skin was stored in the skin banking solution at 4°C and the thickness of this tissue was the same as whole human skin.

Dog buccal mucosa was taken from mongrel dogs immediately after sacrifice. The mucosa was dissected from the cheek tissue of the animals with care being taken to prevent damage to the mucosal tissue. This dissected tissue was nearly 1400 μ in thickness and was

stored in the same banking solution as human and pig skin.

Permeation studies were conducted using the glass cells shown in the Figure. The cell exposes 7.85 cm^2 of tissue and has a volume of 13 ml on each side of the tissue which is mounted between the two glass halves. Adequate mixing of the 13-ml bathing solutions was accomplished by stirring propellers attached to Teflon stirring rods driven by 400 rpm synchronous motors.

The tissue to be studied was placed vertically between the two half chambers with a neoprene O-ring seal on the epithelial surface of the tissue. After the tissue was mounted, phosphate Ringer's solution buffered at pH 7 was added to the dermal side of the tissue. Simultaneously, the same solution containing a radioactively labeled drug or tritiated water (THO) was added to the epithelial side of the tissue. Standards were taken from this donor solution at the beginning and end of the experiment to ensure that the concentration of tracer solute had not decreased during the experiment. Samples were taken at intervals from the solutions bathing the "downstream" side of the membrane after a suitable time was allowed for the isotope to come to steady-state within the skin. Care was taken to ensure that there was no significant buildup of tracer in the receptor side which could lead to a significant backflux of radioactivity. All experiments were conducted in a thermostated bath at 30°C and the cold solute plus tracer solute mixture was prepared by dissolving both in a suitable solvent and recrystallizing the solute. If experiments showed no difference in permeability between solutions prepared this way, and permeability obtained by simply adding both the radioactive species and cold species to the buffer, this latter method of preparing the donor solution was used.

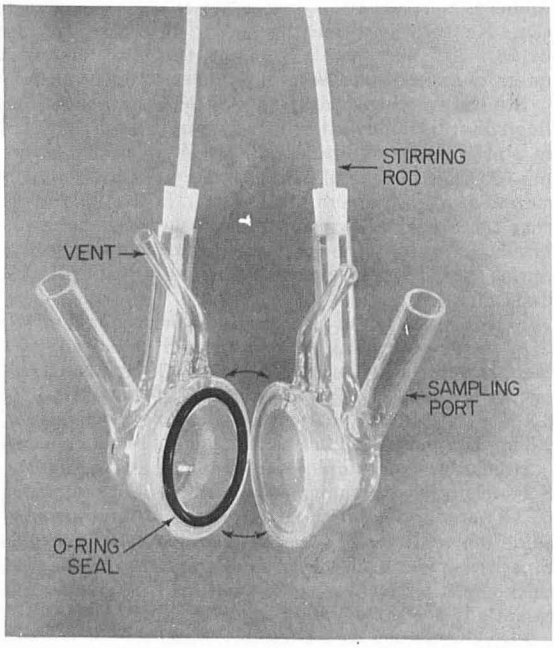

FIG. Chamber assembly. The tissue to be studied is placed between the two chamber halves which are held together by an O-ring seal clamp (not shown). Solutions are placed on the two sides of the membrane and samples taken through the sampling ports. The vents opening at the top of each chamber adjacent to the membrane surface allow for the removal of trapped air bubbles which would otherwise occlude a portion of the membrane surface area. Stirring is accomplished by motors driving the two Teflon stirring rods which are attached to Teflon propellers in the respective chambers.

All radioactively labeled solutes were obtained from New England Nuclear Corp., Boston, Mass., as either the ^{14}C-labeled or ^{3}H-labeled species. The tracer solutes were tested for isotopic purity by paper, column, or thin-layer chromatography prior to and on occasion after experiments to ensure that the penetrating species was indeed whole molecule rather than a radioactively labeled fragment. Radioactive counting was done by liquid scintillation using a Packard 3385 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

The permeability coefficient (P_s) is calculated by dividing the net total transfer of radioisotope during each time interval (dpm/time) by the radioactivity of the donor side solution (dpm/cc) and the area of the membrane exposed for diffusion (cm^2). The resulting value was defined as P_s and has the units of cm/time. A mean of all such intervals and the standard deviation of these values were then calculated. Since no general time trend was seen in the values of P_s, it may be concluded that measurements were taken during steady-state flux.

P_s is related to the total net flux by the equation:

$$P_s = J_s/\Delta C \times A$$

where J_s = the net flux of the solute; ΔC = the concentration difference across the membrane; A = the area of skin exposed to the drug (7.85 cm^2 in our cells).

RESULTS

Table I presents data from a study that was conducted to determine the effectiveness of the preservation medium in maintaining the permeability properties of skin. Initially, fresh skin was tested without preservation for its permeability to THO. The remaining portion of the skin, taken from the same cadaver, was maintained in the preservation medium at 4°C for varying lengths of time and was tested for its permeability to THO. No increase in permeability was observed for periods of storage up to and including 5 weeks. The variations in permeability that were seen probably reflect the differences between the individual pieces of skin; they are not due to skin deterioration, since, in all cases that we have observed, the deterioration of a skin sample is paralleled by increases in skin permeability to THO. At no time did the permeability of the preserved skin exceed that of fresh skin. Furthermore, no consistent differences were found in the P_s values between stored and fresh skin for other solutes. Thus, we conclude that storage in the above-described preservation medium has no significant effect on whole-skin permeability for periods of time as long as 5 weeks.

In all of the studies reported here, the integrity of the skin was assessed by measuring the THO P_s on a portion of each skin specimen in parallel with the other permeability experiments. Whenever the

TABLE I. *Permeability of tritiated water*

Weeks of storage	$P_s \pm$ SD (10^{-7} cm/sec)	No. of experiments[a]
Fresh	5.5 ± 0.7	3
1	4.2 ± 0.6	2
2	4.4 ± 0.8	2
5	3.6 ± 1.4	3

[a] Each experiment consists of 8 to 12 permeability determinations.

TABLE II. *Permeabilities of various tissues to selected molecules*

	No. of experiments[a]	$P_s \pm$ SD (10^{-7} cm/sec)
THO		
Hydrated whole human skin	50	4.4 ± 1.7
Hydrated whole pig skin	4	5.0 ± 0.8
Hydrated human dermis	4	611 ± 83
Dog buccal mucosa[b]	4	511 ± 61
Amphetamine		
Hydrated whole human skin	3	0.039 ± 0.001
Hydrated human dermis	3	265 ± 33
Dog buccal mucosa[b]	3	300 ± 68
Estradiol		
Hydrated whole human skin	6	10.8 ± 3.3
Hydrated human dermis	4	153 ± 52
Dog buccal mucosa[b]	3	132 ± 57
Ouabain		
Hydrated whole human skin	9	0.011 ± 0.006
Hydrated human dermis	5	148 ± 65
Dog buccal mucosa[b]	2	130 ± 46

[a] Each experiment consists of 8 to 12 permeability determinations.

[b] Permeabilities corrected for thickness as described in text.

THO P_s was found to be abnormally large, the experiments using that particular skin specimen were discarded.

The P_s for THO and P_s for each of three drugs are listed in Table II for whole human skin, human dermis, and dog buccal mucosa (corrected for thickness as described below). The permeability of whole pig skin to THO is also presented.

It is immediately apparent that the permeability of human and pig skin are nearly the same for THO. Both of the tissues show permeabilities of approximately 5×10^{-7} cm/sec, which compare favorably with the value of 3×10^{-7} cm/sec found by Scheuplein [8] for human epidermis determined at 25 °C, although our value for the permeability of human skin to estradiol is roughly 10-fold greater than that reported by Scheuplein et al [9].

Also immediately apparent is the fact that the permeability of whole human skin is 14 to as much as 6800 times smaller than the permeability of human dermis. This is a reflection of the fact that the epidermis, and, in particular, the stratum corneum is the rate-limiting barrier to the penetration of most substances [10–12]. Thus, permeabilities of whole human skin can be regarded as those of the epidermis since the dermis obviously is much more permeable than the whole skin.

Somewhat surprising is the excellent agreement between the permeabilties of dog buccal mucosa and that of human dermis. Since the buccal mucosa tissue sheet was generally in the range of 1400 to 1600 μ in thickness while human dermis was in the range of 750 μ thick, the P_s of dermis and of buccal mucosa have been adjusted to a thickness of 750 μ to facilitate comparison. Of course, such an adjustment assumes the tissues to be homogeneous with respect to their permeability properties. This is justified by the observation that two pieces of buccal mucosa of different thicknesses show very nearly identical permeabilities when adjusted to the same thickness. If the permeabilities were determined by a rate-limiting layer or layers within the membrane, a correction of the P_s for thickness would tend to cause divergence in the calculated permeabilities of the two pieces of tissue rather than increase agreement betweem them. The thickness adjustment is accomplished by multiplying the observed P_s by the ratio of the tissue thickness to 750 μ. For instance, a buccal mucosa of 1400-μ thickness and a P_s of 250×10^{-7} cm/sec would have a corrected P_s of 250×10^{-7} cm/sec $\times$ 1400 μ/750 μ = 467×10^{-7} cm/sec.

Data are presented in Table III to emphasize the permeability properties of the three respective tis-

TABLE III. *Permeability of tissues to various solutes*

Solute	$P_s \pm$ SD (10^{-7} cm/sec)	H_2O solubility (mg/ml)	M	$P_s \cdot M^{1/2} \pm$ SD (10^{-7} cm/sec)
	A. Whole Human Skin			
Estradiol	10.8 ± 3.3	4×10^{-3}	273	178 ± 54
Water	4.4 ± 1.7	—	18	18.6 ± 7.2
Amphetamine	0.039 ± 0.001	113	135	0.45 ± 0.01
Ouabain	0.011 ± 0.006	12	585	0.27 ± 0.14
	B. Human Dermis			
Water	611 ± 83	—	18	2600 ± 400
Amphetamine	265 ± 33	113	135	3100 ± 400
Estradiol	153 ± 52	4×10^{-3}	273	2500 ± 900
Ouabain	148 ± 65	12	585	3600 ± 1300
			Mean $P_s \cdot M^{1/2} \pm$ SD =	2900 ± 800
	C. Dog Buccal Mucosa[a]			
Water	511 ± 61	—	18	2200 ± 300
Amphetamine	300 ± 68	113	135	3500 ± 800
Estradiol	132 ± 57	4×10^{-3}	273	2200 ± 900
Ouabain	130 ± 46	12	585	3100 ± 1100
			Mean $P_s \cdot M^{1/2} \pm$ SD =	2800 ± 800

[a] P_s values corrected for thickness as described in text.

sues. In the second column, we see that the very water-insoluble steroid, estradiol, more easily penetrates whole human skin than does water. Ouabain, which is 10-fold less soluble in water than amphetamine, shows a lower permeability than amphetamine. Hence, it can be concluded that the permeability of the skin to these drugs is not directly or inversely related to their water solubility.

One might question this conclusion on the basis that since the diffusional mobility of a molecule in water for this molecular size range is inversely proportional to the square root of its molecular weight ($M^{1/2}$) [13], and since the solutes studied represent a wide range of molecular weights, the permeabilities observed are the resultant of not only chemical characteristics but also their physical sizes. To correct for this effect of molecular size on diffusion, one can multiply the observed P_s by the $M^{1/2}$ of each solute. Thus, P_s values for large molecules are compensated for the decrease in diffusion caused by their greater size and all solutes may be compared equally.

The last column of Table III shows that the relative ordering of whole-skin permeabilities for the solutes studied is not affected by the $M^{1/2}$ correction. We can now conclude that the selectivity of the whole skin membrane is not on the basis of molecular size or lack of water solubility alone. These observations are not surprising since it is widely believed that the membrane/water partition coefficient is a major permeability determining factor for epidermis [11,13,15].

In section *B* of Table III, the permeabilities shown by human dermis are listed. The P_s values are arranged in decreasing order, from water having a P_s values of 6×10^{-5} cm/sec to ouabain and estradiol having P_s values of about 1.5×10^{-5} cm/sec. It is interesting to note that estradiol and ouabain show the same permeability although their water solubilities are different by a factor of 3,000. The permeability coefficients when multiplied by the square root of the molecular weight of the solutes ($P_s \cdot M^{1/2}$) are all seen to be about the same. Statistically, no difference can be seen between the $P_s \cdot M^{1/2}$ values for all the solutes tested. This suggests that the differences seen in the observed P_s values are due mainly to differences in molecular size and that the dermal tissue possesses relatively little, if any, selectivity based on the chemical properties of the solute.

From section *C* of Table III, it is seen that the permeability of buccal mucosa completely parallels that of human dermis. Not only do the relative permeabilities follow, but the absolute magnitudes of the P_s values are practically identical. The values of $P_s \cdot M^{1/2}$ show little evidence for selectivity by the in vitro buccal mucosa to the solutes tested.

Furthermore, the averages of the $P_s \cdot M^{1/2}$ values for human dermis and dog buccal mucosa suggest that the membranes behave in essentially an identical manner.

DISCUSSION

Supplies of human cadaver skin adequate enough to allow extensive permeability studies are sometimes difficult to obtain. It is, therefore, important and timely that we find an excellent correlation between the permeability of human and pig skin to THO in vitro. This observation, combined with the previous observations of Ainsworth [16] who showed a good agreement between the in vitro permeabilities of human and pig skin to tributylphosphate, and the generally good agreement between the in vivo skin permeability of human and pig skin for several organic substances seen by Bartek et al [17], indicate this animal may be an excellent subject for the study of topical drug delivery both in vivo and in vitro.

Our observation that skin stored in the banking solution maintains its THO permeability characteristics for up to 5 weeks and possibly longer further facilitates long or extensive studies which do not require daily procurement of skin samples.

The diffusion coefficient of THO in in vitro human dermis, using the methods described here was found to be 4.5×10^{-6} cm²/sec, in good agreement with the value of 2×10^{-6} cm²/sec reported by Blank and Scheuplein [12] using a somewhat different in vitro method. It seems clear that the permeability of human dermis in vitro is 2 to 3 orders of magnitude greater than either that of the much thinner epidermis or the skin as a whole, consisting of both the epidermis and dermis. It therefore follows that the epidermis is the rate-limiting barrier to the penetration of water through the skin. These studies corroborate the conclusions of previous investigators with respect to the relative permeabilities of these two substructures of the skin to hydrophylic substances [10,12].

For the four penetrants studied, we found no correlation between the permeability of the epidermis to a solute and either the water solubility or the molecular size of the permeating solute. This suggests that some factor other than the diffusibility of the solute in water regulates the permeability of human epidermis to nonelectrolytes. It has been previously postulated that the permeability of the epidermis to solutes is determined by their epidermis/water partition coefficients which in general have been estimated from oil/water partition coefficients. However, since neither oil/water nor epidermis/water partition coefficients have yet been determined for the above solutes, we have not attempted to draw any conclusions with respect to the usefulness of this parameter in determining skin permeabilities.

Our observation that $P_s \cdot M^{1/2}$ is quite constant for all of the studied solutes penetrating dermis and buccal mucosa leads us to conclude that permeation through these tissues is diffusion limited. In other words, the permeation of the tissue by the solute is determined only by the molecular size of the solute species and not by the hydrophobicity or hydrophilicity of the molecule. We conclude from

the aqueous nature of the dermal tissue, the high permeability of the tissue to THO, and the constancy of the $P_s \cdot M^{1/2}$ values that the tissue acts as a large-pored, water-filled membrane by the criteria of Davson and Danielli [18]. This being true, we can compare the product of the apparent diffusion coefficients (D) and the square roots of the molecular weight of these solutes diffusing through dermis to that calculated by Davson and Danielli [18] for solutes in free solution. These investigators have determined the value of $D \cdot M^{1/2}$ for a number of nonelectrolytes in free solution and have shown that it is constant with a value of 7×10^{-5} cm^2/sec. Taking the dermis to be 750 μ thick, we calculate the value of $D \cdot M^{1/2}$ for human dermis to be 2×10^{-5} cm^2/sec using $D = P_s \Delta_x$, where Δ_x is the diffusional barrier thickness (i.e., dermal thickness). From this, we are led to conclude that dog buccal mucosa and human dermis are very similar in their permeability properties, both showing an effective diffusion area of about 30% of the total membrane calculated from $D \cdot M^{1/2}_{(\text{membrane})}/D \cdot M^{1/2}_{(H_2O)}$. As a matter of comparison, the value of $D \cdot M^{1/2}$ for rabbit dermis of 8.8×10^{-6} cm^2/sec as determined by Treherne [19] is about 40% of the value we find for both human dermis and canine buccal mucosa. Thus, although we have found human dermis and canine buccal mucosa to have essentially the same value for $D \cdot M^{1/2}$, and therefore the same effective diffusional areas, this is not universal for all such tissues.

Many of the laboratory procedures described in this paper and a portion of the experimental data reported herein, were developed in the laboratories of ALZA Corp., Palo Alto, California; the authors wish to acknowledge ALZA's authorization to publish this information. The authors also wish to acknowledge the technical assistance of A. Reuter.

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