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Buccal delivery of progestational steroids: I. Characterization of barrier properties and effect of penetrant hydrophilicity

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Summary

In an attempt to characterize the barrier functions of buccal mucosa, a series of systematic studies were performed, using the rabbit as the animal model. The model penetrants used in these studies were progesterone, a lipophilic compound, and the hydroxy derivatives of progesterone, which have approximately similar molecular weights but differ in their hydrophilicity, to study the effect of a systematic variation in lipophilicity on the kinetics and rate of permeation across the rabbit buccal mucosa. The results indicated that transbuccal permeation occurs via both a lipoidal pathway and an aqueous-pore pathway. The mucosal permeability and partition coefficient are both governed by the degree of hydrophilicity, and are increased with removal of the lipid domain from the mucosal membrane.

Introduction

Oral delivery has been the preferred route of drug administration. However, this drug-delivery route has some inherent disadvantages, in that, the drugs are subjected to extensive presystemic elimination, including gastrointestinal degradation/ metabolism and/or hepatic 'first-pass' clearance. This results in low systemic bioavailability, short duration of therapeutic activity, and/or inactive or even toxic metabolites of the drug.

Parenteral delivery of drugs that are subjected to variable absorption, due to their susceptibility to the gastrointestinal environment, represents a viable alternative. Drugs administered via the parenteral route can gain direct access to the systemic circulation, resulting in total bioavailability in most cases. However, this mode of drug administration may entail certain health risks, such as physical and psychic pain, occasional allergies, and hypertrophy or atrophy of the subcutaneous fat at the injection site, especially for injection on a chronic basis, e.g., the daily subcu-

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taneous injection of insulin in the therapy of diabetes. (Ishida et al., 1981).

Systemic delivery of drugs through the mucosal routes presents a solution to the problems of hepatic and gastrointestinal metabolism associated with the oral drug-delivery route, and the health risks associated with the parenteral route.

The buccal mucosa is an easily accessible and convenient site for drug delivery, whence hepato-gastrointestinal first-pass elimination of drugs is avoided. It also has the advantages of being a robust mucosa due to routine exposure to food and other 'foreign substances', and increased patient compliance (Veillard et al., 1987; Veillard and Antony, 1990; De Vries et al., 1991).

To achieve systemic delivery of drugs by absorption via the buccal mucosa, it is important to have some insight into the barrier functions of the buccal mucosa and the effect of physicochemical properties of drugs on buccal absorption. Although the nature of the barrier function of the buccal mucosa has been extensively reviewed from a physiological standpoint (Wertz and Squier, 1991), a survey of the literature indicated that to date there are only few systematic studies conducted to evaluate the effect of physicochemical properties of drugs on their buccal absorption (Garren and Repta, 1989; Le Brun et al., 1989). It has already been established earlier that the permeability barrier, in the case of the buccal mucosa, lies in the upper one-third region of the membrane (Squier and Hall, 1985).

To establish a relationship between the physicochemical properties of a penetrant molecule and the mechanism and kinetics of buccal absorption, transbuccal permeation kinetic studies were performed, using progesterone and a series of its hydroxy derivatives (Fig. 1) with systematic variation in hydrophilicity, as model penetrants.

Materials and Methods

Materials

Progesterone, 11α-hydroxyprogesterone, 11β-hydroxyprogesterone, 17α-hydroxyprogesterone, 21-hydroxyprogesterone, 11β,21-dihydroxyprogesterone (corticosterone), and 17α,21-dihydroxyprogesterone (cortexolone), and 11β,17α,21-trihydroxyprogesterone (hydrocortisone) were used as received (Sigma Chemical Co., St. Louis, MO). Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride, polyethylene glycol 400, and methanol were all reagent grade and used as obtained (Fisher Scientific, Fair Lawn, NJ). The constituents of a modified artificial saliva (Shellis, 1978) were also used as supplied.

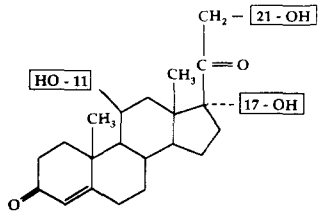

Fig. 1. Chemical structure of progesterone and its hydroxy derivatives, indicating the various potential sites for hydroxy group attachment.

Sample preparation

Female New Zealand white rabbits (Davidson's Mill Farm, Jamesburg, NJ), weighing about 7–8 lb, were killed by i.v. injection of sodium pentobarbital (150 mg/kg) just prior to the experiments. The buccal mucosa was surgically removed, the underlying fat and connective tissue were carefully cleaned, and the mucosa rinsed briefly with normal saline (Veillard et al., 1987; Gandhi and Robinson, 1991).

Physical characteristics of the rabbit buccal mucosa

The thickness, percent unbound water, percent extractable lipid and percent protein were determined for at least six mucosal samples. Mucosal thickness was measured by removing the underlying connective tissue and muscle from the freshly excised mucosa, placing between two slides, and measuring the thickness of the composite using a calliper, with and without the mucosa. The percent unbound water was estimated from the difference in the weight of the mucosal samples, which had been cleaned off the connective tissue and muscle, immediately after excision ($\sim$ 60 mg) and after drying at 45°C for 48 h. To estimate the percent lipid content of the mucosa,

freshly excised mucosae (~ 60 mg each) were cleaned as before and placed in a solution of chloroform : methanol (2 : 1), with shaking, for 2 h (Corbo et al., 1990). The chloroform : methanol extract was then evaporated under a stream of nitrogen, and the extracted lipids weighed.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to qualitatively assess the interactions between the lipid and protein domains in the intact and delipidized buccal mucosa. Freshly excised and delipidized mucosal samples were placed in hermetically sealed metal DSC capsules, which minimized water interference, and were scanned using a differential scanning calorimeter (Perkin Elmer model DSC-7, Norwalk, CT) from 10 to 180°C.

Solubility studies

It had been previously determined that a time period of 12 h was sufficient to achieve saturation solubility. The solubility of the drugs in artificial saliva with and without 20% PEG 400, added as a solubilizer for progesterone and its hydroxy derivatives, was determined by equilibrating an excess amount of progesterone or its hydroxy derivatives in artificial saliva at 37°C for 24 h, filtering the solution through a teflon filter (0.45 μm, which was preheated to 37°C), and the filtrate obtained was analyzed by HPLC.

In vitro permeation studies

The in vitro permeation kinetic studies were conducted in hydrodynamically well-calibrated Valia-Chien permeation cells (Crown Glass Co., Sommerville, NJ) (Chien and Valia, 1984). Each permeation cell consisted of one pair of half-cells in mirror image, with each having a volume of 3.5 ml and a surface area of 0.64 cm^2 for transmucosal permeation. The permeation of tritiated water across the mucosa was also studied in order to ensure the physical integrity of the mucosal membrane over the study period. An increase in the permeation rate of tritiated water would indicate damage to the mucosa. Since this was not observed, the integrity of the mucosa was concluded to be maintained for 24 h.

The same procedure as developed by Corbo et al. (1990), to study transmucosal permeation kinetics across nasal, vaginal and rectal mucosae was applied. Briefly, freshly excised buccal mucosae were each cleaned and mounted over the opening between the half-cells with the mucosal epithelium facing the donor half-cell. The donor half-cell contained a saturated solution of progestational steroid (3.5 ml), which had been previously equilibrated with excess steroid for at least 12 h prior to the experiment to achieve saturation concentration, in artificial saliva (at pH 6.8) containing 20% PEG 400. The receptor half-cell contained 3.5 ml of isotonic phosphate buffer (at pH 7.4), also containing 20% PEG 400 to maintain sink conditions on the serosal side to mimic the active hemoperfusion under physiological conditions.

For permeation studies on the delipidized mucosa, freshly excised mucosa was mounted on the Valia-Chien cell and the solution of chloroform : methanol (2 : 1) was placed in the donor and the receptor compartments. After extraction for 2 h, the solution was aspirated and any remaining traces evaporated under a gentle stream of nitrogen (Corbo et al., 1990). The donor and receptor compartments were then filled, respectively, with the donor and receptor solutions outlined earlier and permeation studies carried out.

Samples (300 μl each) of the receptor solution were withdrawn at preset time intervals and replaced with fresh (drug-free) receptor solution.

The donor cell concentrations were determined at the end of the 24 h study and found to be similar to the saturation solubility.

Mucosal partition coefficient studies

The mucosal partition coefficient studies were carried out by placing freshly excised and cleaned, intact or delipidized mucosa (~ 60 mg each) in a known volume of the respective donor solution containing a fixed concentration of each steroid, and equilibrating the solutions in a shaking water bath at 37°C for a period of 24 h. Control experiments were also run (without the mucosae) to ensure the thermal stability of the steroids over the duration of partition studies. The amount of steroid partitioning into the mucosa was calcu-

lated from the reduction in steroid concentration in the solution at the end of the 24 h partitioning study and was used to calculate the concentration of steroid in the mucosa. The mucosal partition coefficient was then determined from the concentration in the mucosa over that in the solution at equilibrium.

Analytical method

The samples were assayed using a HPLC system (Waters pump model 590 and Waters intelligent sample processor model 712) and a μBondapak C-18 column (3.9 mm × 150 mm) from Waters (Milford, MA), together with a Kratos Spectroflow 773 detector (Spectra-Physics, San Jose, CA) at a wavelength of 240 nm. The mobile phase consisted of a mixture of methanol:water at different ratios for progesterone and its various hydroxy derivatives, at a flow rate of 1.5 ml/min. The HPLC assay developed had a detection limit of 0.1 μg/ml.

Data analysis

The rate of buccal permeation was determined from the mucosal permeation profile. From the permeation rate obtained, permeability and diffusivity were calculated using the solubility and partition coefficient values determined experimentally in separate studies.

Triplicate experiments were run for each study and the results are reported as mean values (±1 S.D.).

Results and Discussion

Physical characteristics of the rabbit buccal mucosa

The rabbit buccal mucosa has a lower extractable lipid content (3.8%) but a higher protein content (19.9%) than the nasal, rectal and vaginal mucosae excised from the same animal (Corbo et al., 1990). The buccal mucosa (0.10 ± 0.009 cm) is thicker than the nasal mucosa (0.05 ± 0.008 cm) but thinner than the rectal (0.125 ± 0.014 cm) and vaginal (0.15 ± 0.018 cm) mucosae. The percent unbound water in the rabbit buccal mucosa is 76.3 (±4.3%) which is very much the same as that in the other mucosae.

DSC

DSC serves as a qualitative tool to assess the interactions between the protein and lipid domains in the biological membranes. The DSC scans of the intact and delipidized buccal mem-

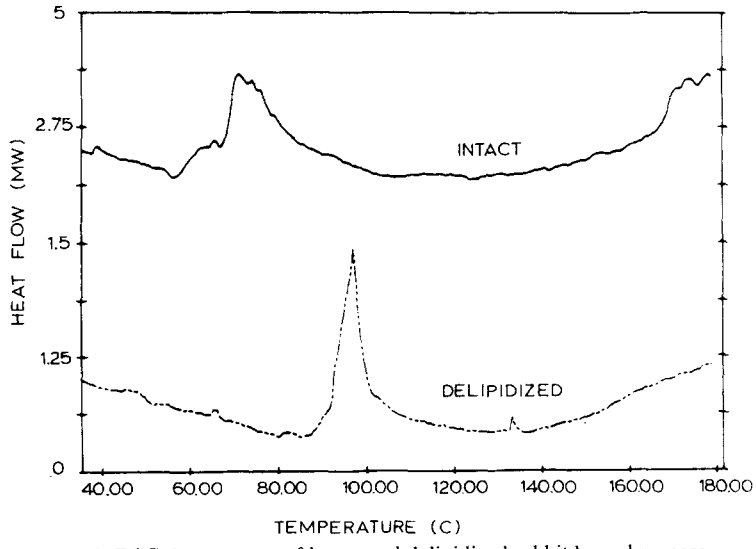

Fig. 2. DSC thermogram of intact and delipidized rabbit buccal mucosa.

branes are shown in Fig. 2. In the case of the intact mucosa, lipid-protein interactions are observed to occur in the range of 60–90°C. In the case of the delipidized mucosa, there is a change in the thermal transition peaks, with a sharp peak appearing at high temperature (around 95°C). This may have resulted from the disorganization of the protein regions in the mucosa caused by the delipidization, leading to a change in the barrier properties of the mucosa (Knutson et al., 1985).

In vitro permeation studies

Permeation of model lipophilic compound Progesterone was chosen as a model penetrant on the basis of its lipophilicity. The permeation of progesterone across the rabbit buccal mucosa was observed to follow zero-order kinetics after a lag time of approx. 6 h (Fig. 3), as expected from Eqn 1.

$$(Q/t)_b = P_b(C_d - C_r) \quad (1)$$

where $(Q/t)_b$ denotes the rate of transbuccal permeation, P_b is the apparent permeability across the buccal mucosa, and $(C_d - C_r)$ represents the difference in drug concentrations between the donor and receptor solutions.

If conditions exist such that $C_d \gg C_r$, Eqn 1 can be reduced to

$$(Q/t)_b = P_b C_d \quad (2)$$

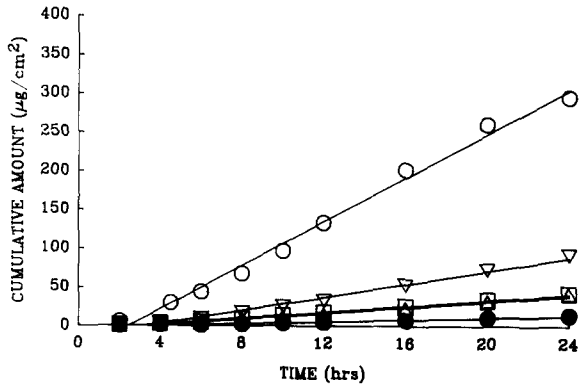

Fig. 3. Effect of hydroxy group position on the permeation profile of monohydroxyprogesterone across rabbit buccal mucosa ($n = 3$, ±S.D.). (●) Progesterone (P); (□) 11β-OH-P; (△) 17α-OH-P; (▽) 11α-OH-P; (○) 21-OH-P.

and the apparent permeability (P_b) can be calculated according to

$$P_b = (Q/t)_b / C_d \quad (3)$$

The rate of permeation is determined from the linear portion of the permeation profile for each drug (Eqn 2), and the resulting value is then divided by the donor concentration to obtain the mucosal permeability coefficient (Eqn 3).

Effect of hydroxy group position As seen in Fig. 1, the hydroxy group in monohydroxyprogesterone can be added at either position 11 (α or β), 17(α), or 21 around the steroid nucleus. The results in Fig. 3 indicate that the zero-order ki-

TABLE 1

Effect of hydroxy group position on buccal permeation

Progestin	Permeation parameters [a]			Solubility [a,b] (μg/ml) (±S.D.)
	Lag time (h) (±S.D.)	Rate (μg/cm² per h) (±S.D.)	Permeability (cm/h) (±S.D.) (×10²)	
Prog. (P)	6.10 (±0.27)	0.80 (±0.12)	1.34 (±0.18)	59.6 (±0.8)
11α-OH-P	4.25 (±0.27)	6.18 (±0.12)	2.08 (±0.04)	297.7 (±37.1)
11β-OH-P	3.75 (±0.19)	2.09 (±0.07)	1.65 (±0.05)	126.5 (±1.1)
17α-OH-P	5.61 (±0.62)	2.22 (±0.43)	2.32 (±0.44)	95.9 (±2.1)
21-OH-P	2.74 (±0.58)	14.4 (±0.74)	2.71 (±0.14)	532.0 (±23.5)

[a] Mean (±1 S.D.) of three determinations.
[b] Artificial saliva (pH 6.8) with 20% PEG 400.

netics of transbuccal permeation is not affected by the addition of a hydroxy group, although the rate of permeation changes. The extent of change in permeation rate depends on the site of hydroxy group addition on the progesterone skeleton. As can be seen from Fig. 3, addition of a hydroxy group at position 21 results in a considerable increase in permeation rate. The permeation rate also appears to depend on the stereochemical configuration of the hydroxy group, as indicated by the 3-fold greater permeation of 11α-hydroxyprogesterone as compared to its 11β analogue (Table 1). However, this higher rate of transbuccal permeation observed for 11α- and 21-hydroxyprogesterone could be attributed to their greater aqueous solubility in the artificial saliva (Table 1).

The apparent permeability can be calculated using Eqn 3. The results obtained are also outlined in Table 1, which suggests that addition of one hydroxy group increases the permeability of progesterone by up to 2-fold.

Effect of penetrant hydrophilicity More than one hydroxy group can be incorporated into the progesterone nucleus resulting in dihydroxy and trihydroxy derivatives of progesterone. Fig. 4 demonstrates the effect of successive addition of hydroxy groups at the three possible positions. The addition of up to three hydroxy groups on the progesterone molecule does not alter the zero-order kinetics of permeation but affects the rate of transbuccal permeation. In the case of the monohydroxy and dihydroxy derivatives, the degree of effect observed is proportional to the solubility of each derivative and depends on the number and the position of the hydroxy groups added. In the case of trihydroxyprogesterone, however, the rate of permeation decreases in spite of its higher solubility. (Table 2)

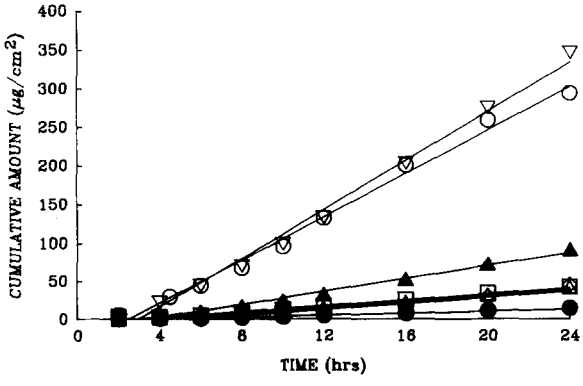

Fig. 4. Effect of number of hydroxy groups on the permeation profile of hydroxyprogesterone across rabbit buccal mucosa. ($n = 3$; ±S.D.). (●) Progesterone (P); (□) 11β-OH-P; (△) 17α-OH-P; (◇) 11β,17α,21-tri-OH-P; (▲) 17α,21-di-OH-P; (○) 21-OH-P; (▽) 11β,21-di-OH-P.

TABLE 2

Permeability of progesterone and its hydroxy derivatives across rabbit buccal mucosa

Progestins	Solubility [a,b] (μg/ml) (±S.D.)	Permeability [a] (cm/h) (±S.D.) ($\times 10^2$)
Progesterone (P)	59.6 (±0.8)	1.34 (±0.18)
Mono-OH-P		
11β-	126.5 (±1.1)	1.65 (±0.05)
17α-	95.9 (±2.1)	2.32 (±0.44)
21-	532.0 (±23.5)	2.71 (±0.14)
Di-OH-P		
11β,21-	566.7 (±40.6)	3.12 (±0.03)
17α,21-	142.5 (±11.3)	3.38 (±0.21)
Tri-OH-P		
11β,17α,21-	1018.4 (±5.1)	0.28 (±0.05)

[a] Mean (±1 S.D.) of three determinations.
[b] Artificial saliva (pH 6.8) with 20% PEG 400.

The permeabilities of the derivatives were calculated using Eqn 3. The results are summarized in Table 2, which indicate that the permeability across rabbit buccal mucosa increases with the addition of one hydroxy group and further increases with addition of the second hydroxy group. However, on addition of the third hydroxy group, the permeability decreases substantially.

Effect of delipidization The role of lipid domain in maintaining the barrier function of the buccal mucosa was studied by extracting the lipids in the mucosa with chloroform : methanol (2 : 1) for 2 h. Delipidization caused no change in the zero-order transbuccal permeation kinetics but substantially altered the permeation rate of progesterone and its hydroxy derivatives. The comparison in Fig. 5a demonstrates that while the buccal mucosal permeability of progesterone, a lipophilic penetrant, is reduced on delipidization, that of its hydroxy derivatives is in all cases en-

hanced. The results clearly suggest that the more the number of hydroxy groups added to the progesterone molecule, the greater the enhancement in mucosal permeability resulting from delipidization (Fig. 5b).

In Fig. 5b, the $\log(P_{\text{delipidized}}/P_{\text{intact}})$ value for the rabbit buccal mucosa increases linearly with an increase in the number of hydroxy groups added to the progesterone molecule.

Mucosal partition coefficient

Intact mucosa The rate of transbuccal permeation is dependent upon the apparent permeability and the difference in concentration between the donor and receptor solutions, as indicated in Eqn 1. The apparent permeability is in turn determined by the mucosal partition coefficient (K_m), the mucosal diffusion coefficient (D_m)

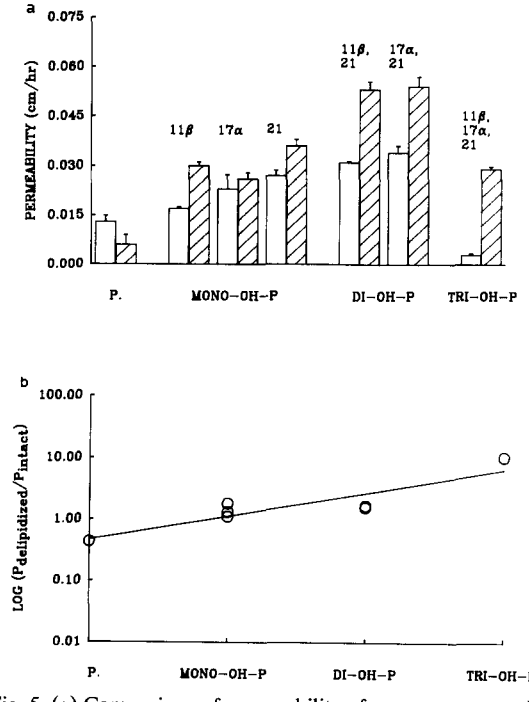

Fig. 5. (a) Comparison of permeability of progesterone and its hydroxy derivatives across intact (□) and delipidized (▨) rabbit buccal mucosa ($n = 3$; ±S.D.). (b) Exponential dependence of the ratio of permeability across delipidized and intact buccal mucosae of rabbit ($P_{\text{delipidized}}/P_{\text{intact}}$) on the number of hydroxy groups on the progesterone molecule.

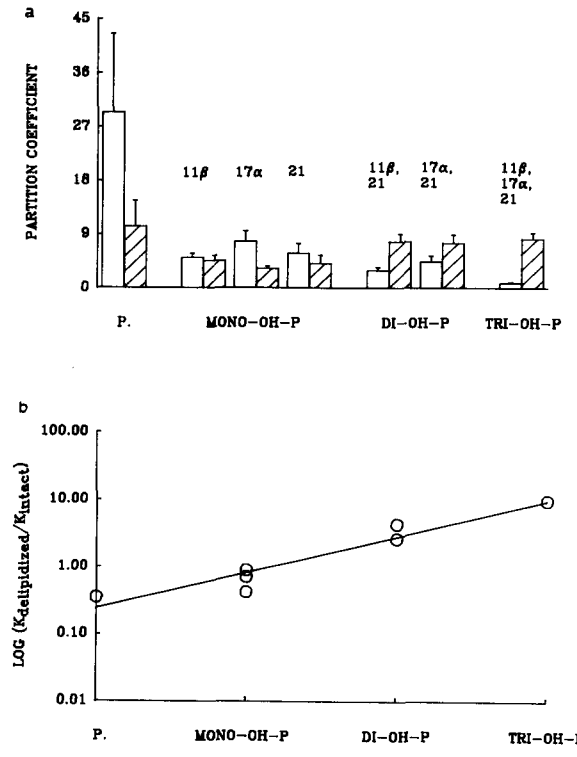

Fig. 6. (a) Comparison of partition coefficient of progesterone and its hydroxy derivatives across intact (□) and delipidized (▨) rabbit buccal mucosa. ($n = 3$; ±S.D.). (b) Exponential dependence of the ratio of partition coefficient across delipidized and intact buccal mucosae of rabbit ($K_{\text{delipidized}}/K_{\text{intact}}$) on the number of hydroxy groups on the progesterone molecule.

and the mucosal membrane thickness (h_m) according to

$$P_b = K_m D_m / h_m \tag{4}$$

To investigate the effect of mucosal lipophilicity on buccal absorption, the partition coefficients of progesterone and its hydroxy derivatives were determined in the buccal mucosa-artificial saliva system. The results summarized in Fig. 6a indicate that the partition coefficients for the interfacial partitioning of progestational steroids from the artificial saliva to the buccal mucosa decrease with the number of hydroxy groups added to the progesterone molecule.

Delipidized mucosa Delipidization of the mucosa results in a considerable alteration of the

mucosal partition coefficient. Upon delipidization, the partition coefficient of progesterone, a lipophilic molecule, was observed to decrease considerably. On the other hand, the mucosal partition coefficient of hydroxyprogesterone was slightly reduced for the monohydroxy derivative but was increased for the di- and trihydroxy derivatives (Fig. 6a).

Fig. 6b shows that the $\log(K_{\text{delipidized}}/K_{\text{intact}})$ value for the rabbit buccal mucosa increases linearly with increase in the number of hydroxy groups added to the progesterone molecule.

The alteration in the mucosal partition coefficients of progesterone and its hydroxy derivatives on delipidization is in good agreement with the variation in mucosal permeabilities (Figs 5b and 6b). The agreement suggests that the enhancement in the mucosal permeability of progestational steroids resulted from the increase in mucosal partitioning. In other words, delipidization increases the partition coefficient of hydroxy progesterone, especially of the di- and trihydroxy derivatives, and thus their mucosal permeabilities.

The results in Figs 2 and 6a and b suggest that the removal of extractable lipids altered the mucosal partitioning of progesterone and its hydroxy derivatives, which could result from the shift from a lipoidal domain to a protein domain; therefore, the permeability across the delipidized mucosa increases with the number of hydroxy groups added (Fig. 5a and b).

Effect of mucosal diffusivity

Mucosal diffusivity is to some extent related to the molecular size of the penetrants. To determine the effect of molecular weight on permeation, the diffusivity of progesterone and its hydroxy derivatives was calculated for both intact and delipidized rabbit buccal mucosa using the following relationship derived from Eqn 4,

$$D_m = P_b h_m / K_m \qquad (5)$$

The results summarized in Table 3 indicate that for the intact buccal mucosa, the mucosal diffusivity increases as the number of hydroxy groups added increases up to two hydroxy groups and then decreases for the trihydroxy derivative.

TABLE 3

Diffusivity of progesterone and its hydroxy derivatives across rabbit buccal mucosa

Progestins	Diffusivity [a] (cm^2/h) ($\pm$S.D.) ($\times 10^5$)	
	Intact mucosa	Delipidized mucosa
Progesterone (P)	4.7 ($\pm$0.62)	5.7 ($\pm$2.76)
Mono-OH-P		
11β-	32.5 ($\pm$1.83)	65.4 ($\pm$2.41)
17α-	29.9 ($\pm$5.74)	79.2 ($\pm$5.83)
21-	47.0 ($\pm$2.42)	88.0 ($\pm$4.96)
Di-OH-P		
11β,21-	106.0 ($\pm$1.13)	68.0 ($\pm$3.38)
17α,21-	76.9 ($\pm$4.84)	71.7 ($\pm$4.42)
Tri-OH-P		
11β,17α,21-	33.4 ($\pm$5.76)	35.3 ($\pm$0.95)

[a] Mean ($\pm$1 S.D.) of three determinations.

Following delipidization, the mucosal diffusivity for monohydroxyprogesterone increases 2-fold compared to that for intact buccal mucosa. The diffusivity of progesterone and its trihydroxy derivative shows only a very slight increase, while that of dihydroxyprogesterone decreases slightly.

In view of the fact that the addition of hydroxy groups has only a slight influence on the molecular weight of progesterone (only 5% increase for every hydroxy group added), the alteration in mucosal diffusivity observed (Table 3) could be attributed to the effect of hydroxy groups on the interaction of the progesterone molecule with the mucosal tissue. Addition of hydroxy groups could decrease the penetrant-mucosa interactions, leading to an increase in mucosal diffusivity. This effect is complex and appears to be dependent upon the number and position of hydroxy groups added as well as the biochemical composition of the mucosal tissue.

Conclusions

Absorption of drugs through mucosae has been reported to occur via both a transcellular (lipoidal) pathway and an intercellular (aqueous pore)

route (Powell, 1981; Chien, 1990; Corbo et al., 1990). The same has been theorized regarding the buccal mucosa (De Vries et al., 1991; Chien, 1992). This was demonstrated to be the case as judged from the results obtained in this investigation with the rabbit buccal mucosa. On delipidization, the transbuccal permeability of progesterone was observed to decrease but that of its hydroxy derivatives progressively increased with monohydroxy, dihydroxy and trihydroxy derivatives, thus substantiating the pathways proposed above.

The exponential increase in the ratio (delipidized mucosa/intact mucosa), of the permeability as well as of the partition coefficient, with increase in the number of hydroxy groups could indicate that following delipidization, the permeation of hydroxyprogesterone through the intercellular pathway is enhanced as a result of the reduction in diffusional resistance by eliminating the extractable lipids in the intercellular space.

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