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Enhanced delivery of ketobemidone through porcine buccal mucosa in vitro via more lipophilic ester prodrugs

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Summary

The in vitro penetration of ketobemidone and various ester prodrugs through porcine buccal mucosa in a modified Ussing chamber was investigated in order to support the selection of a prodrug derivative with optimal buccal absorption. The nine esters studied included carboxylic acid and carbonate esters formed at the phenolic hydroxy group of ketobemidone The esters were all rapidly hydrolyzed to the parent drug in a porcine buccal epithelial homogenate and only free ketobemidone was detected in the receptor compartment of the Ussing chamber. All the ester prodrugs showed enhanced rates of permeation relative to ketobemidone, the permeability coefficients being 3–30-times higher than that of ketobemidone The permeability coefficients increased with increasing lipophilicity, expressed in terms of octanol-buffer (pH 7.4) partition coefficients (P), up to log P values of about 1.5 whereupon a plateau or a slight decrease occured No toxic effects of ketobemidone or the prodrugs on the buccal membrane were observed as judged from monitoring of the electrical properties of the membrane.

Introduction

The buccal and sublingual routes of administration represent an attractive alternative to peroral administration of drugs undergoing extensive first-pass metabolism in the intestine or the liver as the veins from the oral cavity bypass the liver. Furthermore, these routes offer a potential for more rapid absorption and onset of action relative to the peroral and rectal routes. Recent studies have shown that the bioavailability of various drugs such as opioid analgesics and opioid antagonists can be greatly improved by buccal or sublingual administration (Bullingham et al., 1982; Bell et al., 1985; Hussain et al., 1986, 1987, 1988; Weinberg et al., 1988).

Ketobemidone (I) is a strong narcotic analgesic which is equipotent with morphine (Eddy et al., 1957; Anderson et al., 1986). Due to an extensive and variable first-pass metabolism ketobemidone shows an incomplete and variable bioavailability following peroral and rectal administration to humans (Bondesson et al., 1980; Anderson et al., 1981, 1982). Initial studies using the in vivo buccal absorption model of Beckett and Triggs (1967) showed that ketobemidone did not penetrate the human oral mucosa which most likely can be ascribed to its low lipophilicity (Hansen et al.,

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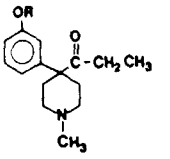
1991). This physico-chemical property is known to be an important parameter in the penetration of drugs across the oral mucosal barrier (Pickup and Beckett, 1977; Siegel, 1984; Kurosaki et al., 1986; Weinberg et al., 1988, Garren and Repta, 1989; Le Brun et al., 1989; Rathbone, 1991).

With the aim of improving the buccal absorption of ketobemidone we have recently prepared a number of carboxylic acid and carbonate ester prodrugs at the phenolic hydroxyl group in the drug (Hansen et al., 1991). These derivatives were found to be more lipophilic than the parent drug and to be rapidly and quantitatively hydrolyzed to ketobemidone in the presence of human plasma (Hansen et al., 1991). Furthermore, their resistance to undergo a saliva-catalyzed hydrolysis under clinically relevant conditions was found to be so high that this factor would not be expected to compromise buccal absorption of the prodrugs to any marked extent (Hansen et al., 1992b).

The objective of the present study was to examine the in vitro transport characteristics of ketobemidone and various ester prodrugs across the porcine buccal mucosa in a modified Ussing chamber in order to support the selection of a prodrug derivative with optimal buccal absorption. The ketobemidone esters (**II–X**, Scheme 1) studied cover a wide range of lipophilicity and the investigation was therefore also believed to give useful information on the influence of lipophilicity on permeability. The non-keratinized porcine buccal tissue was chosen, since it has been shown to be a good model for the human buccal tissue (Squier and Hall, 1985).



Compound	Name	R
I	Ketobemidone	-H
II	O-Acetyl ketobemidone	$-COCH_3$
III	O-Pivalyl ketobemidone	$-COC(CH_3)_3$
IV	O-3,3-Dimethylbutyryl ketobemidone	$-COCH_2C(CH_3)_3$
V	O-Benzoyl ketobemidone	$-COC_6H_5$
VI	O-Methoxycarbonyl ketobemidone	$-COOCH_3$
VII	O-Ethoxycarbonyl ketobemidone	$-COOC_2H_5$
VIII	O-Isopropoxycarbonyl ketobemidone	$-COOCH(CH_3)_2$
IX	O-Isobutoxycarbonyl ketobemidone	$-COOCH_2CH(CH_3)_2$
X	O-Butoxycarbonyl ketobemidone	$-COOC_4H_9$

Scheme 1 Chemical structure of ketobemidone and various esters investigated in this study

Materials and Methods

Chemicals and apparatus

Ketobemidone hydrochloride was obtained from H. Lundbeck A/S, Copenhagen, Denmark. The ketobemidone esters **II–X** were prepared as previously described (Hansen et al., 1991). The ester **II** was isolated as the fumarate salt whereas all the other esters were hydrochloric acid salts. A glucose bicarbonate Ringer solution (GBR) (pH 7.4) with the following composition was used in the permeation study: Na^+, 141 mM; K^+, 5 mM; Ca^{2+}, 1.2 mM; Mg^{2+}, 1.2 mM; Cl^-, 122 nM; HCO_3^-, 25 mM; HPO_4^{2-}, 1.6 mM; $H_2PO_4^-$, 0.4 mM, and glucose 10 mM. In the modified Ussing chamber (Ussing, 1949) measurements of the electrical properties of the membrane were carried out in a current clamp set-up equal to that used by Bindslev (1979).

Diffusion experiments

Buccal tissue was removed from pigs at the abattoir, immediately after death and was transported in oxygenated GBR solution at 0°C. Prior to mounting in the Ussing chambers or to homogenization the connective tissue was carefully excised using a pair of scissors and a tissue slicer.

During the diffusion experiments open-circuit transepithelial potential differences (PD) and short-circuit current (I_{SC}) were monitored. Electrical resistance (R) and conductance (G) were calculated using Ohm's law.

Mounting the tissue in the Ussing chambers was completed within 90 min from the death of the animals. The tissue was bathed on both sides by 10.0 ml of GBR solution. The bathing solutions were circulated by gas lift with 95% O_2/5% CO_2 which also provided oxygenation (bubbling

rate, 3–4 bubbles/s) and maintained at 37°C by water-jacketed reservoirs.

Preliminary studies showed that the potential difference and the short-circuit current stabilized within 60 min after mounting the tissue. Therefore, the tissue was preincubated with GBR solution for 60 min prior to addition of drug to the mucosal half-chamber. 2–4 ml of GBR solution facing the mucosal half-chamber was then removed and instantly replaced with an equal volume of GBR solution containing the drug or prodrug to be studied. The initial concentration of the compounds in the mucosal half-chamber was equivalent to 1.0 mg of ketobemidone hydrochloride per ml. Samples of 1 ml were withdrawn from the serosal half-chamber at appropriate times and were replaced with drug-free GBR solution. The samples were centrifuged at 10000 rpm for 10 min and analyzed by reversed-phase HPLC.

The steady-state fluxes (F_{SS}) were calculated from the slopes of the linear portion of plots of cumulative amount of permeated ketobemidone (in μg ketobemidone base) per unit surface area vs time of sampling. The permeability coefficients (K_P) for the steady-state permeation were calculated by dividing the steady-state fluxes by the drug or prodrug concentration in the donor solution.

The influence of various diffusion apparatus related parameters on drug permeation was evaluated in preliminary experiments. Thus, the influence of the electrical conditions, i.e., open-circuit vs short-circuit conditions, was investigated. Furthermore, the possible appearance of edge damage of the tissue was evaluated by comparing the permeability coefficients obtained when employing chambers with different diameter openings (0.64 and 0.95 cm^2). Also, the intra- and inter-subject variation of porcine buccal mucosa was examined. The study was performed as a factorial experiment.

None of the parameters tested were found to significantly influence the permeability. Hence, it was decided to carry out the subsequent experiments using open-circuit conditions, chambers with a diameter opening of 0.95 cm^2 and mucosa from different pigs in each chamber.

Hydrolysis in buccal mucosa homogenate

The hydrolysis of the ketobemidone esters was studied in porcine buccal epithelial homogenate (2% w/v dry buccal epithelium in 0.05 M phosphate buffer of pH 7.4) at 37°C. The buccal epithelial homogenate was prepared according to the method previously described for human skin homogenate (Hansen et al., 1992a). The initial concentration of the compounds was 3×10^{-5} M. The reactions were initiated by adding 50 μl of a stock solution of the esters in water to 2.5 ml of preheated homogenate. The mixtures were kept in a water-bath at 37°C and at appropriate intervals, 250 μl samples were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in methanol-water (1:1 v/v) to deproteinize the samples. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed for intact ester and ketobemidone by HPLC as described below. All experiments were carried out in duplo.

Analysis of ketobemidone and its esters by HPLC

Ketobemidone and the various esters were determined by reversed-phase HPLC procedures as previously described (Hansen et al., 1991). The detection limit for ketobemidone was about 0.1 $\mu g\ ml^{-1}$.

Results and Discussion

Hydrolysis in buccal mucosa homogenates

The susceptibility of the ketobemidone esters to undergo enzymatic hydrolysis by buccal epithelial enzymes was studied at 37°C in a 2% w/v porcine buccal epithelium homogenate (pH 7.40). The rates of hydrolysis of all esters were found to follow first-order kinetics at an initial concentration of 3×10^{-5} M, as illustrated in Fig. 1 for some esters. In all cases, the hydrolysis of the esters in the homogenates proceeded with the quantitative formation of ketobemidone as illustrated in Fig. 2. The observed half-lives for the hydrolysis are listed in Table 1. As it appears from the data, the esters are easily hydrolyzed by the mucosal enzymes. In pure buffer solutions at pH 7.4 and 37°C, the half-lives of hydrolysis of

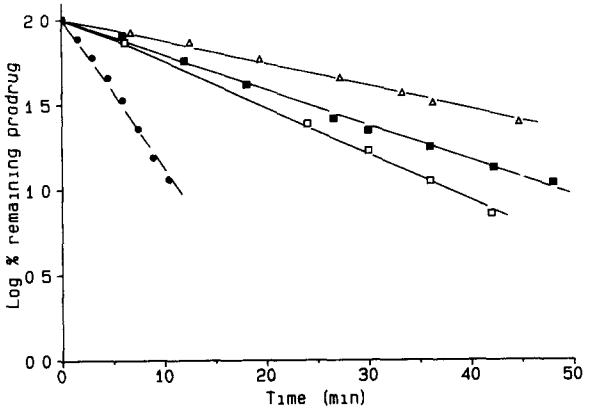


Fig. 1 First-order plots for the hydrolysis of the ketobemidone esters **II** (■), **IV** (△), **VIII** (□) and **IX** (●) in 2% w/v porcine buccal epithelium homogenate (pH 7.40) at 37°C

the compounds are in the range 50–1000 h whereas in 80% human plasma half-lives of 0.03–1.8 min have been found (Hansen et al., 1991).

It is of interest to note that the sterically hindered pivalate (**III**) and 3,3-dimethylbutyrate (**IV**) ester are hydrolyzed at rates only slightly lower than that for the acetate ester (**II**) and more rapidly than the methyl carbonate ester (**VI**).

Permeability studies

The cumulative amounts of ketobemidone that had penetrated through the porcine buccal epithelium is shown in Fig. 3 for ketobemidone and some ester prodrugs. After a lag-time of 1–2 h, a linear relation was found in all experiments. For

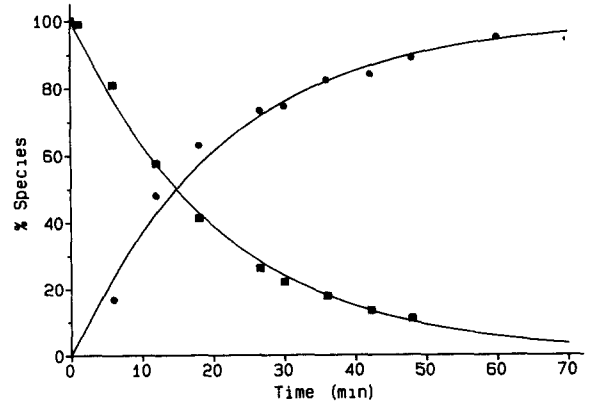


Fig. 2 Time courses for compound **II** (■) and ketobemidone **I** (●) during hydrolysis of the prodrug derivative in 2% w/v porcine buccal epithelium homogenate (pH 7.40) at 37°C The initial prodrug concentration was 3×10^{-5} M

TABLE 1

Steady-state fluxes (F_{SS}), permeability coefficients (K_P), partition coefficients (P) and half-lives of hydrolysis of ketobemidone and various ketobemidone esters

Compound	F_{SS} [a] (μg/cm^2 per min)	K_P ($\times 10^4$) (cm/min)	log P [b]	$t_{1/2}$ [c] (min)
I	0.044 ± 0.025	0.51 ± 0.29	0.40	
II	0.15 ± 0.071	1.6 ± 0.81	0.43	14.4
III	1.19 ± 0.36	13.6 ± 4.2	2.11	19.5
IV	0.85 ± 0.28	9.7 ± 3.3	2.55	21.4
V	0.99 ± 0.29	11.4 ± 3.4	2.41	4.8
VI	0.46 ± 0.33	5.2 ± 3.8	0.57	26.6
VII	1.07 ± 0.36	12.3 ± 4.1	1.11	11.6
VIII	1.35 ± 0.67	15.5 ± 7.7	1.54	11.5
IX	0.94 ± 0.55	10.8 ± 6.3	2.20	2.8
X	1.07 ± 0.045	12.3 ± 0.52	2.24	5.0

[a] The compounds diffused through porcine buccal epithelium from aqueous GBR solution (pH 7.4) at equimolar concentrations corresponding to 1.0 mg ml^{-1} of ketobemidone hydrochloride. Mean ± S.D (n = 3–4).

[b] P: partition coefficient between octanol and 0.05 M phosphate buffer of pH 7.4 at 22°C (from Hansen et al., 1991)

[c] Half-lives for hydrolysis in a 2% porcine buccal epithelial homogenate (pH 7.4) at 37°C

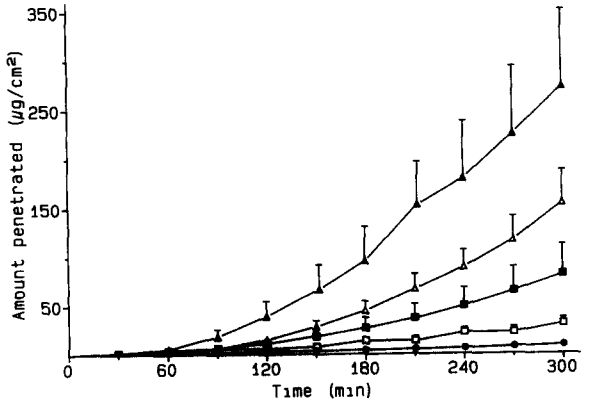


Fig. 3. Permeability of ketobemidone **I** (●) and the ketobemidone esters **II** (□), **IV** (△), **VI** (■) and **VIII** (▲) through porcine buccal epithelium expressed as the amount of ketobemidone appearing in the receptor phase as a function of time The compounds were applied as aqueous solutions (pH 7.4) at equimolar concentrations corresponding to 1.0 mg ml^{-1} of ketobemidone hydrochloride. Error bars represent standard errors of the mean values (S.E.) (n = 4)

all esters only ketobemidone was detected in the receptor solution, indicating an efficient enzymatic hydrolysis of the esters during the transport through the buccal membrane. This finding is in accordance with the short half-lives of hydrolysis observed in the epithelial homogenate.

The steady-state fluxes and permeability coefficients obtained are listed in Table 1 along with the octanol-pH 7.4 aqueous buffer partition coefficients.

As can be seen from the data, all prodrugs tested showed higher fluxes and permeability coefficients than the parent drug, the enhancement being in the range 3–30. A plot of the permeability coefficients vs the log P values of the compounds (Fig. 4) shows that the permeability increases with increasing lipophilicity up to a log P value of about 1.5 whereafter it remains almost constant up to a log P value of 2.5. The data show a tendency to a parabolic dependence of penetration on lipophilicity, which cannot, however, be assessed with certainty from the data available. The results confirm the important role of lipophilicity in the passive drug transport across various biomembranes including the buccal epithelium. At low log P values the diffusion across the lipoidal epithelial membrane is the rate-determining step whereas in the plateau region the rate-determining step becomes diffusion across the aqueous boundary layer (Ho et al., 1992). Le Brun et al. (1989) have previously shown that the in vitro permeability of various β-blocking agents of porcine buccal mucosa increases with increasing lipophilicity of the compounds, the log P values for the compounds studied ranging from -0.6 to 1.3.

Electrical properties of the buccal membrane

Porcine buccal mucosa when freshly mounted in the Ussing chamber had a short-circuit current (I_{SC}) of 7–20 μA/cm^2, a transepithelial potential differenses (PD) of 10–25 mV with the lumen negative and a transepithelial conductance (G) of 0.3–0.95 mS/cm^2. The low transepithelial conductance (i.e., high resistance) indicates an electrically 'tight' tissue. Both I_{SC} and PD declined with time. Thus, after incubation for 60 min in GBR solution the porcine buccal mucosa showed

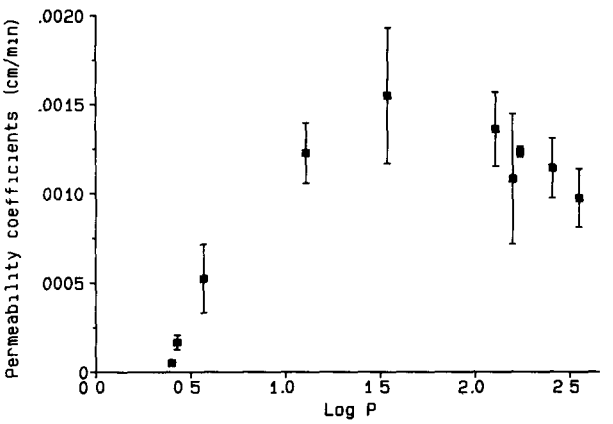


Fig. 4. Permeability coefficients (K_P) for ketobemidone and various ketobemidone esters through porcine buccal epithelium as a function of their octanol-buffer pH 7.4 partition coefficients (log P). Error bars represent standard errors of the mean values (S.E.) ($n = 3$–4)

a potential difference of 10–25 mV, a short-circuit current of 5–12 μA/cm^2 and a conductance of 0.3–0.7 mS/cm^2. At the end of the study, i.e, after 5 h, the potential difference was found to be 5–17 mV and the short-circuit current to be 1–7 μA/cm^2. These findings are in agreement with those of Orlando et al. (1988) who determined electrical parameters of dog buccal mucosa after equilibration for 45 min in Ussing chamber to be I_{SC} 15 ± 1 μA/cm^2, PD -16 ± 2 mV, mean R 1090 ± 100 Ω cm^2 and mean G of 1.06 ± 0.08 mS/cm^2. No differences in the electrical properties of the porcine buccal mucosa were seen when no drug, ketobemidone or the prodrug derivatives were present at the mucosal side in the Ussing chambers, indicating no destructive effects of the compounds on the membrane in the concentration used.

Conclusions

The results obtained in the present study show that the permeation of ketobemidone through porcine buccal mucosa can be markedly improved by using ester prodrugs with a higher lipophilicity and a high susceptibility to undergo conversion to the parent drug via mucosal esterases. Neither the ester prodrugs nor ketobemidone itself seem

to exhibit local toxic effects on the porcine buccal membrane as judged by monitoring the electrical properties of the membrane.

The results of an in vivo buccal absorption study of the prodrugs in rats are described in the following paper (Hansen et al., 1992c).

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References

Anderson, P., Arnér, S., Bondesson, U., Boréus, L.O. and Hartvig, P., Clinical pharmacokinetics of ketobemidone. Its bioavailability after rectal administration. *Eur J Clin Pharmacol.*, 19 (1981) 217–223.

Anderson, P., Arnér, S., Bondesson, U., Boréus, L.O and Hartvig, P., Single-dose kinetics and bioavailability of ketobemidone. *Acta. Anaesth. Scand.*, 74, Suppl., (1982) 59–62.

Anderson, P., Arnér, S., Bondesson, U., Boréus, L.O. and Hartvig, P., Pharmacokinetics of ketobemidone. In Foley, K.M. and Inturrisi, C.E (Eds), *Advances in Pain Research and Therapy*, Vol 8, Raven, New York, 1986, pp. 171–178.

Beckett A.H and Triggs E.J., Buccal absorption of basic drugs and its application as an in vivo model of passive drug transfer through lipid membranes *J. Pharm. Pharmacol.*, 19 (Suppl.) (1967) 31S–41S

Bell, M D.D., Mishra, P., Weldon, B.D., Murray, G.R., Calvey, T.N and Williams, N.E., Buccal morphine – a new route for analgesia? *Lancet, I* (1985) 71–73.

Bindslev, N., Sodium transport in the hen lower intestine. Induction of sodium sites in the brush border by a low sodium diet *J Physiol.*, 288 (1979) 449–466

Bondesson, U., Arnér, S., Anderson, P., Boréus, L.O. and Hartvig, P., Clinical pharmacokinetics and oral bioavailability of ketobemidone. *Eur J Clin Pharmacol.*, 17 (1980) 45–50.

Bullingham, R.E.S., McQuay, H.J., Porter, E.J.B., Allen, M.C. and Moore, R.A., Sublingual buprenorphine used postoperatively: Ten hour plasma drug concentration analysis *Br J. Clin. Pharmacol.*, 13 (1982) 665–673.

Eddy, N.B., Halbach, H. and Braenden, O.J., Synthetic substances with morphine-like effect. Clinical experience: potency, side-effects, addiction liability. *Bull. WHO*, 17 (1957) 569–863.

Garren K.W. and Repta A J., Buccal drug absorption II: In vitro diffusion across the hamster cheek pouch *J Pharm Sci.*, 78 (1989) 160–164.

Hansen, L B., Christrup, L L. and Bundgaard, H., Ketobemidone prodrugs for buccal delivery *Acta Pharm Nord.*, 3 (1991) 77–82

Hansen, L.B., Fullerton, A., Christrup, L.L and Bundgaard, H., Enhanced transdermal delivery of ketobemidone with prodrugs *Int J Pharm.*, 84 (1992a) 253–260

Hansen, L B., Christrup, L L and Bundgaard, H., Ketobemidone prodrugs for buccal delivery· Prediction of the extent of saliva-catalyzed hydrolysis of various ester prodrugs under simulated in vivo conditions *Int J Pharm.*, 88 (1992b) 229–235

Hansen, L B., Jørgensen, A., Rasmussen, S N., Christrup, L L and Bundgaard, H., Buccal absorption of ketobemidone and various ester prodrugs in the rat *Int J Pharm.*, 88 (1992c) 243–250

Ho, N F H., Barsuhn, C L., Burton, P S. and Merkle, H P., Mechanistic insights to buccal delivery of proteinaceous substances. *Adv Drug Del Rev.*, 8 (1992) 197–235.

Hussain, M A., Aungst, B.J., Kearney, A and Shefter, E., Buccal and oral bioavailability of naloxone and naltrexone in rats *Int J Pharm.*, 36 (1987) 127–130

Hussain, M A., Aungst, B J., Koval, C A. and Shefter, E., Improved buccal delivery of opioid analgesics and antagonists with bitterless prodrugs *Pharm Res* 5 (1988) 615–618

Hussain, M A., Aungst, B J. and Shefter, E., Buccal and oral bioavailability of nalbuphine in rats *J Pharm Sci.*, 75 (1986) 218–219

Kurosaki, Y., Aya, N., Okada, Y., Nakayama, T and Kimura, T., Studies on drug absorption from oral cavity Physicochemical factors affecting absorption from hamster cheek pouch. *J Pharmacobio-Dyn.*, 9 (1986) 287–296.

Le Brun, P P H., Fox, P L A., De Vries, M.E and Boddé, H E., In vitro penetration of some β-adrenoreceptor blocking drugs through porcine buccal mucosa *Int J Pharm.*, 49 (1989) 141–145.

Orlando, R.C., Tobey, N.A., Schreiner, V.J and Readling, R.D., Active electrolyte transport in mammalian buccal mucosa *Am. J Physiol.*, 18 (1988) G286–G291

Pickup, M E. and Beckett, A H., Steroid absorption – use of the substituent constant π' *J Pharm Pharmacol.*, 29 (1977) 715–716.

Rathbone, M J., Human buccal absorption. II A comparative study of the buccal absorption of some parahydroxybenzoic acid derivatives using the buccal absorption test and a buccal perfusion cell. *Int J Pharm.*, 74 (1991) 189–194.

Squier, C A and Hall, B K., The permeability of skin and oral mucosa to water and horseradish peroxidase as related to the thickness of the permeability barrier. *J Invest Dermatol.*, 84 (1985) 176–179.

Siegel I.A., Permeability of the rat oral mucosa to organic solutes measured in vivo *Arch Oral Biol.*, 29 (1984) 13–16

Ussing, H H., The active ion transport through the isolated frog skin in the light of tracer studies. *Acta Phys Scand.*, 17 (1949) 1–37

Weinberg, D S., Inturrisi, C.E., Reidenberg, B., Moulin, D E., Nip, T J., Wallenstein, S., Houde, R W. and Foley, K.M., Sublingual absorption of selected opioid analgesics *Clin. Pharmacol Ther.*, 44 (1988) 335–342