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# Recovery of the rat vaginal epithelium from the histological effects of absorption enhancers

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## Summary

The recovery of the rat vaginal epithelium from the histological effects of absorption enhancers was assessed 24 h after treatment with laureth-9 and lysophosphatidylglycerol (LPG) solutions. In a control group of rats, the vaginal epithelium had a regular appearance and showed no sign of damage 24 h after administration of buffer solution. The vaginas treated with 0.5% LPG solution were similar to those of the control group although small sites showing reduced epithelial height were occasionally found. In contrast, 24 h after treatment with 1% laureth-9 solution, severe and widespread epithelial cell loss was evident with few signs of cellular regeneration and recovery. As previous studies have demonstrated absorption enhancing activity of both compounds, these results show that absorption enhancement is not necessarily linked with mucosal damage.

The vagina has potential for the systemic delivery of therapeutic peptide and protein drugs, particularly for those used in the treatment of female-related conditions (Richardson and Illum, 1991). However, in common with other mucosal sites, the vaginal absorption of high molecular weight peptides and proteins, which are typically hydrophilic, is normally low. Consequently, absorption enhancers have been used to improve the bioavailability of peptides administered vaginally (Okada et al., 1982). One of the major concerns in the use of absorption enhancers is their potential local and systemic toxicity, particularly if intended for use in chronic drug therapy.

The effect of a range of enhancers on the vaginal absorption of drugs and the acute histological effects of the enhancer formulations have been studied in ovariectomised and oestradiol-treated rats (Richardson et al., 1989, 1991). Surface-active enhancers laureth-9, lysophosphatidylcholine (LPC) and palmitoylcarnitine chloride (PCC) markedly increased the vaginal absorption of gentamicin in ovariectomised rats but all induced significant epithelial cell loss (Richardson et al., 1989). The thin vaginal epithelium of the ovariectomised rat was particularly susceptible to drug-induced damage, and therefore, further studies were performed in oestradiol-treated, ovariectomised rats, which were considered to be

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a more suitable model (Richardson et al., 1991). In these experiments, laureth-9, LPC, PCC and LPG significantly enhanced the vaginal absorption of insulin but induced varying histological changes in the vaginal epithelium. While vaginal administration of insulin and LPG solution resulted in slight damage, to the surface epithelium alone, more severe changes affecting the deeper cell layers, were evident after treatment with the other enhancers.

In the present study, the histology of the rat vaginal epithelium was assessed 24 h after treatment with buffer or enhancer solutions in order to determine the possible recovery from the epithelial damage seen previously. Laureth-9 and LPG were selected as examples of two effective absorption enhancers displaying differing histological effects when used vaginally. Laureth-9 is a non-ionic surfactant widely used to enhance the absorption of peptides and proteins from mucosal sites but its use may be limited because it induces significant mucosal damage (O'Hagan and Illum, 1990). In contrast, the absorption promotion characteristics of the lysophospholipid, LPG, do not appear to be associated with epithelial disruption and focal mucosal damage.

Consequently, it was of interest to extend the study of the histological effects of LPG in a further group of rats. In addition, the method of administration of the vaginal solutions was modified in order to more closely resemble the situation likely to be encountered when drug formulations are administered vaginally to women. Saturated cotton tampons were chosen as a practical dosage form in that they reduced leakage of the solutions from the vaginal tract, could be used without adhesives and consequently prevented pain in the animals during recovery.

L-$\alpha$-Lysophosphatidylglycerol (LPG) and polyoxyethylene-9-lauryl ether (laureth-9) were purchased from Sigma Chemical Co. (Poole, Dorset) and dissolved in phosphate buffer, pH 7.3, at concentrations of 0.5 and 1.0% w/v, respectively. 17-$\beta$-Oestradiol (Sigma) was dissolved in arachis oil at a concentration of 100 $\mu$g/ml.

Female Wistar rats (Sutton Bonnington, U.K.) weighing approx. 200 g were bilaterally ovariectomised under halothane anaesthesia and allowed to recover for at least 2 weeks. 24 h prior to drug treatment, ovariectomised rats, weighing 250–300 g, were injected subcutaneously with 100 $\mu$l of oestradiol solution (10 $\mu$g/animal).

Three groups of rats ($n = 4$, 8 and 8) were treated with buffer, 0.5% LPG and 1% laureth-9 solution, respectively. The animals were anaesthetised with halothane (May and Baker, Dagenham, Kent) and placed supine on a heated table. Anaesthesia was maintained using halothane supplied by a face mask. The vaginal instillates were administered by means of saturated cotton swabs comparable to that used in rectal studies by Reid and Thomas (1990). The swabs (12 mm in length) were mounted on fine plastic tubes and were soaked in the buffer or enhancer solutions before administration. Saturation of the cotton swabs was achieved by injection of 300 $\mu$l of solution along the length of the tube every 30 min. After 2 h, the swabs were removed and the vaginas were flushed with saline before the rats were allowed to recover. 24 h later, the animals were killed by an overdose of pentobarbitone sodium (Sagatal, May and Baker). The vaginas were removed and placed in Bouin Hollande fixative before processing by conventional steps for light microscopy.

The effects of the buffer and enhancer solutions on the rat vaginal mucosa are shown in Figs 1–3. In the control group, the vaginal epithelium consisted of a basal cell layer covered by several squamous cell layers (Fig. 1). Slight interanimal variations in epithelium thickness were observed but there were no signs of cellular damage after treatment with the buffer solution.

24 h after administration of 0.5% LPG solution, the vaginal mucosa (Fig. 2) was similar in appearance to that of the control group. In one of the eight specimens, the thickness of the vaginal epithelial was reduced to two to three cell layers in some areas and one site was bare of epithelium. However, in the remaining specimens, there was no sign of enhancer-induced damage.

In contrast, severe epithelium desquamation was evident in the region exposed to the cotton swab with 1% laureth-9 solution 24 h after treatment (Fig. 3). Sites either denuded of epithelium or with an epithelium of reduced height were

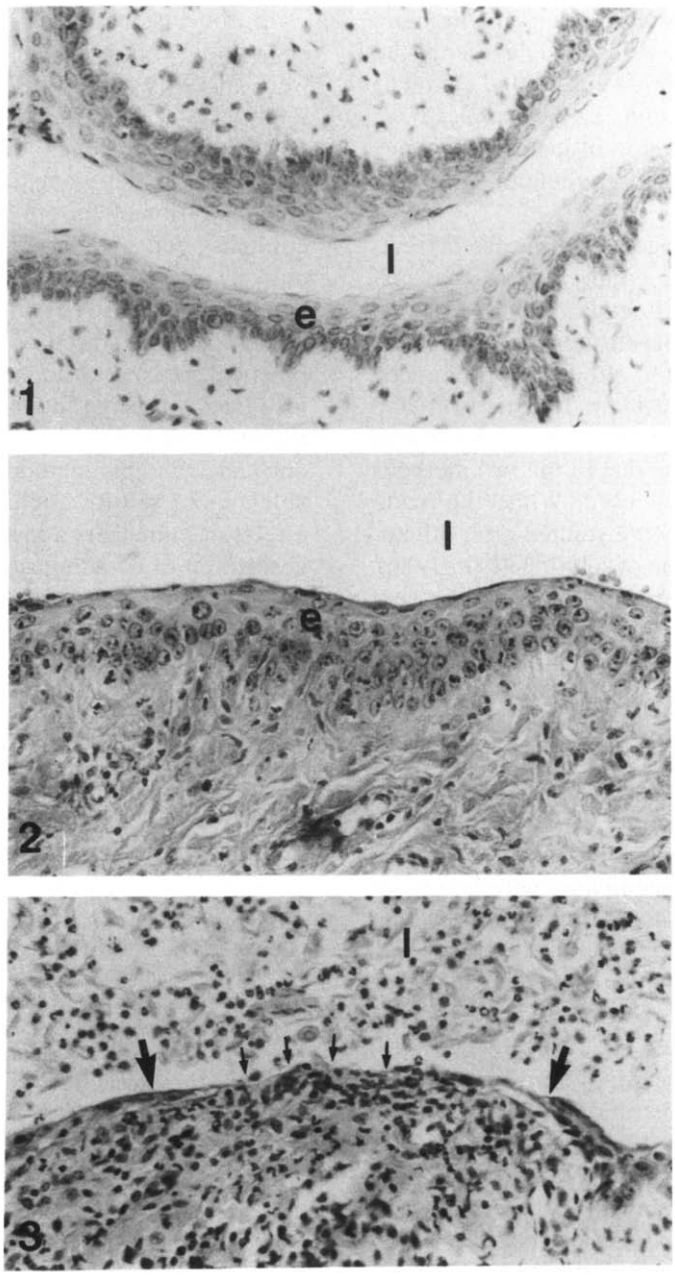


Fig. 1. Photomicrograph of the stratified squamous epithelium (e) from the vagina of a control rat. Note the gradual change in shape from cuboidal to squamous as the layers approach the lumen (l). ×265.

Fig. 2. Photomicrograph of the vaginal epithelium (e) following LPG treatment. The epithelium is composed of approx. the same number of cell layers as the control tissue in Fig. 1. l, lumen. ×265.

Fig. 3. Photomicrograph of the vaginal epithelium following treatment with 1% laureth-9. The lumen (l) is filled with an exudate containing cells and cellular debris. The small arrows mark a region denuded of epithelium while the large arrows indicate sites where the epithelium is one or two cells thick. ×265.

characteristic of this tissue. The lumen contained desquamated cells and cellular debris and the subepithelial connective tissue exhibited evidence of an inflammatory reaction. Tissue samples from sites distant from the region of insult were characterised by an unchanged epithelium similar to that of the control group.

The histological changes resulting from treatment with laureth-9 were more severe than expected. In the previous study, the rat vaginal epithelium remained largely intact 2–4 h after treatment with laureth-9 although pronounced cellular changes were evident (Richardson and Illum, 1991). The differences between the results of the two studies may be due to the two methods of vaginal administration used. While the acute effects of the enhancer were studied after administration of a fixed volume of solution (400 $\mu$l/kg), the use of a saturated cotton swab in the present experiment may have improved the contact of the formulations with the vaginal mucosa and increased the potential for enhancer-induced damage. Obviously, the use of cotton swabs (alone) could not have added to the epithelial damage observed, as the control group of rats showed no signs of mucosal disruption. It is worth noting, that despite the improved contact, LPG showed very little damage when compared with laureth-9.

The poor recovery of the rat vaginal epithelium following laureth-9 treatment is in agreement with the results of studies in which the enhancer was administered nasally to rats (Hirai et al., 1981; Daugherty et al., 1988). In the latter study, epithelial damage was reported to be severe after nasal administration of 1% laureth-9 solution for 6 h to rats (Daugherty et al., 1988). Examination of the nasal mucosa 24 h after this treatment revealed a similar histopathology to that seen after 6 h and confirmed that the epithelial changes induced by the surfactant were not readily reversed.

Similarly, in earlier experiments by Hirai et al. (1981), the recovery of the rat nasal mucosa from histological changes observed 2 h after treatment with laureth-9 was not complete by 24 h. In addition, an enhanced nasal absorption of insulin was evident for several h after treatment with laureth-9 and reversal of this hyperabsorptive state took over 24 h.

In conclusion, the histological effects of two absorption enhancers on the vaginal epithelium were investigated in oestradiol-treated, ovariectomised rats. LPG was confirmed as a promising novel enhancer which did not cause epithelial disruption or desquamation. In contrast, vaginal administration of laureth-9 resulted in widespread epithelial cell loss from which recovery seemed unlikely and certainly was not evident 24 h after treatment. The use of cotton swabs, which mimics some of the features of a tampon, provided a practical method of administration of the vaginal instillates, ensuring intimate contact between enhancer formulations and the vaginal mucosa without leakage. This method of delivery should enable a more sensitive detection of the histological effects of enhancers and should also promote the absorption of co-administered drugs.

## References


Daugherty, A.L., Ligget, H.D., McCage, J.G., Moore, J.A. and Paton, J.S., Absorption of recombinant methionyl human growth hormone (met-hGH) from rat nasal mucosa. *Int. J. Pharm.*, 45 (1988) 197–206.

Hirai, S., Yashiki, T. and Mima, H., Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.*, 9 (1981) 173–184.

O'Hagan, D.T. and Illum, L., Absorption of peptides and proteins from the respiratory tract and the potential for development of locally administered vaccines. *CRC Crit. Rev. Ther. Drug Carr. Syst.*, 7 (1990) 35–97.

Okada, H., Yamazaki, I., Ogawa, Y., Hirai, S., Yashiki, T. and Mima, H., Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats. I: Absorption by different routes and absorption enhancement. *J. Pharm. Sci.*, 71 (1982) 1367–1371.

Reid, A.S. and Thomas, N.W. Crypt microdissection of frozen sections for estimating metaphase counts in the rat rectum following surface desquamation and restitution. *J. Pathol.*, 161 (1990) 354A.

Richardson, J.L. and Illum, L., The vaginal route of peptide and protein drug delivery. *Adv. Drug Del. Rev.*, (1991) in press.

Richardson, J.L., Minhas, P.S., Thomas, N.W. and Illum, L., Vaginal administration of gentamicin to rats. Pharmaceutical and morphological studies using absorption enhancers. *Int. J. Pharm.*, 56 (1989) 29–35.

Richardson, J.L., Illum, L. and Thomas, N.W., Vaginal absorption of insulin in the rat. Effect of penetration enhancers on peptide uptake and mucosal histology. *Pharm. Res.*, (1991) submitted.