In vitro models of mucosal HIV transmission

To the editor—Collins *et al*. published in your April issue a report of an *in vitro* model showing transmission of human immunodeficiency virus (HIV) to cells of the female genital tract[1]. Using a model in which cervical tissue was polarized in 3% agarose, Collins *et al*. reported that within 24 hours, up to 30% of cell-free virus added to the apical surface of the epithelium passed through the tissue. Given what is known about the ultrastructure and permeability of the cervical epithelium, such data are unexpected. The validity of epithelium-containing tissue culture model for transport studies hinges on the presence of tissue confluent in such a way that inoculating virus can gain access to tissue only through the epithelium and not through undetected pores or unsealed edges of the tissue. No convincing data were provided to show what controls were used to eliminate the possibility of such access. Pores, or gaps in the sealing process, however small, can provide access to the underlying stromal tissue and would allow virus to circumvent potential barrier effects of the epithelium. Collins *et al*. used blue dextran (molecular mass, 2×10^6 daltons) as a control to detect defects in sealing around edges of the polarised tissue. However, this does not rule out the possibility of the presence of pores or gaps that would allow passive transport of smaller molecules.

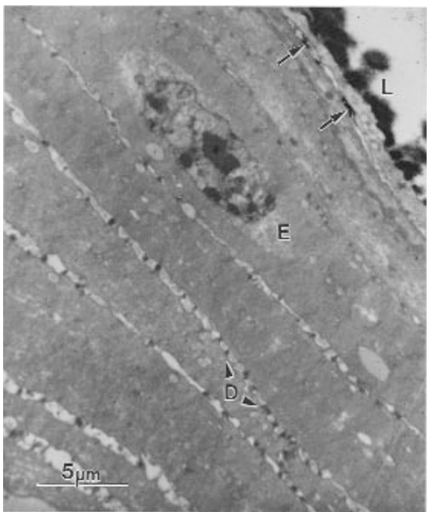

Fig. 1 Ectocervix squamous epithelium labeled during fixation with the intercellular marker lanthanum nitrate. This shows the luminal surface (L) with lanthanum deposits and a limited intercellular permeation of label (arrows). Desmosome bridges (D) join together epithelial cells (E) with the extracellular spaces filled by amorphous glycosaminoglycan material.

Previous studies on tissue from non-human primates have demonstrated that cervical epithelium is impermeable to the small tracer molecules lanthanum nitrate (433 kDa in molecular mass) and horseradish peroxidase[2]; this is also true of human cervical tissue (Fig. 1). Furthermore, intact cervical epithelium, mechanically sealed in an Ussing chamber, is impervious to the passive diffusion of cell-free or cell-associated HIV (ref. 3). Collins *et al*. provided no explanation for the rapid transport of free virus across cervical epithelium in their model. In agreement with extended studies[2], the possible involvement of epithelial infection or transcytosis in viral transmission was excluded. The effect reported by Collins required infectious virus, and inactivated HIV was not readily transmitted. The rapid kinetics of transmission of cell-free virus (maximal in the first 24 hours) indicate it is unlikely to depend on *de novo* infection. Furthermore, possible cross-linking of proteins after inactivation of HIV by ultraviolet irradiation and psoralen could reduce the amount of p24 released from treated virus.

Two possible mechanisms for such rapid transmission of the virus remain. For the first mechanism, the virus is picked up by resident Langerhans and T cells within the epithelium, which subsequently migrate through the tissue and release virus from the basolateral surface. Although epithelial Langerhans and T cells within the epithelium may bind virus and rapidly migrate through tissue, such a mechanism is unlikely to account for transport of up to 30% of the input virus. This is confirmed by the authors' observation that dendritic cell makers remained unchanged throughout the culture period.

In the alternative mechanism, virus can cross the epithelium by passive diffusion. Passive diffusion of virus (80–100 nm in size) through the paracellular pathway would seem unlikely given the results of previous studies, unless integrity of the epithelium had been damaged before experimentation, or paracellular permeability had been artifactually increased. This could occur during surgical removal of or obtaining punch biopsies, known to change the size of intercellular spaces within the epithelium[4]; washing of tissue, which may remove both protective mucous and superficial protective cells[5]; loss of integrity of suprabasal layers, known to contribute to cervical paracellular resistance[6]; incorporation of cholera toxin, known to modify epithelial permeability, into the culture medium; or inefficient sealing of the epithelium with agarose.

Collins *et al*. suggested their observation that transmission of the virus occurred in 70% of examined tissue was in keeping with an estimated *in vivo* transmission rate of 0.01%. An alternative interpretation of this figure would be an expectation that *in vitro* transmission would only occur in 1% of the tissue examined. Thus the possibility remains that only the 30% of the tissue reported to exclude virus was in fact appropriately sealed.

Finally, although the authors attempted to determine the primary target of infection in their model, the use of labeled oligonucleotides to detect HIV *gag–pol* RNA makes it impossible to distinguish whether positive cells are productively infected or merely binding virus on their surface.

Although the reported model is a promising development, more work is needed before it can be accepted as a credible model to study transmission of HIV in the female genital tract. In the meantime, it would be premature to extrapolate the findings from this model to the *in vivo* situation.

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Gupta et al. reply—Shattock *et al*. raised concerns about whether the transmission seen in our organ culture was due