

DIFFUSION OF FLUORESCENT COMPOUNDS INTO MUCUS USING FLUORESCENCE MICROSCOPY AND IMAGE ANALYSIS

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The passage of high molecular weight compounds across mucosal membranes has extensively been studied. Mucus gel layers cover all mucosal membranes and are potential barriers to absorption of such compounds. A rapid method was developed and used to determine the barrier function of the mucus layer.

Fluorescent microscopy (Nikon,UK.) was used to visualise compounds diffusing into mucus gels. The images were captured in an image framestore, then manipulated by software based around Semper 6+ image analysis kernel (Synoptics, UK.). This simple technique allowed diffusion to be followed over very short distances and times.

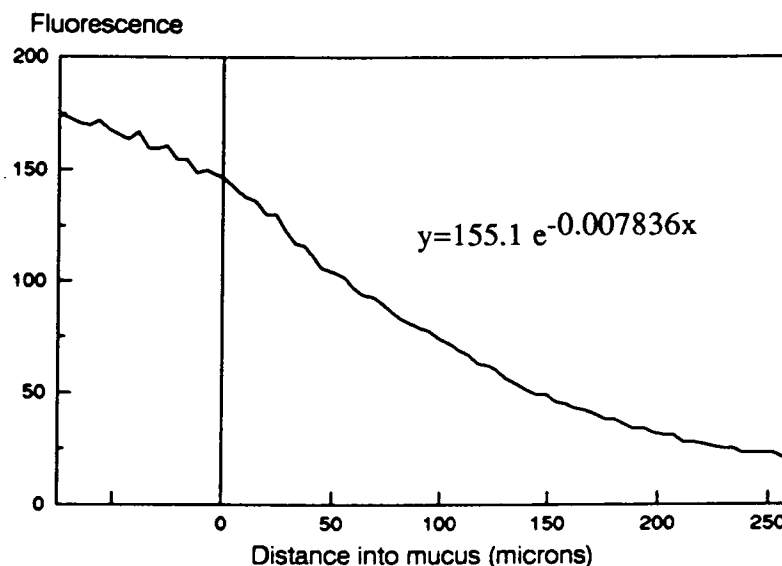
Mucus scraped from pig small intestine, was washed with phosphate buffered saline (PBS) and centrifuged. The mucus plug was centrifuged into hollow microslides (Camlab, UK. Depth 0.4mm) to give an even packing with well defined gel/air interface. FITC-Dextran (fluorescein labelled dextran, Sigma, UK. Relative Molecular Mass [RMM] 35,600) free of unbound fluorescein was dissolved in PBS (1mg/mL). 300 μ L of this solution was placed onto the interface. Diffusion was followed by grabbing images every 15 s for 10 min; under these conditions photo-bleaching was minimal. A field along the diffusional axis was extracted from each picture and corrected for background and uneven illumination. The response of the microscope, camera and analysis program was calibrated using fluorescein (BDH, UK.) standards in PBS.

A linear response is obtained in the range 0-30 μ L/mL for fluorescein. Figure 1. is the penetration profile for FITC-Dextran into mucus after one minute, this has been shown to be exponential ($r=0.9989$) within the gel.

Previous workers have shown that globular proteins of RMM 17,000 cannot enter the mucus gel, suggesting protection of epithelia against enzymic attack (Allen A. 1981).

This work shows that linear molecules of high RMM can penetrate a mucus gel of physiological dimensions.

Figure 1 Penetration of FITC-Dextran into Mucus after 1 Min.



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Allen A. (1981) Basic Mechanisms of GI. Mucosal Cell Injury and Protection. Williams & Wilkins, Ed. J.W. Harmon, London.