GROWTH AND CHARACTERISATION OF A HUMAN COLONIC MUCIN SECRETING CELL LINE HT29-18N2

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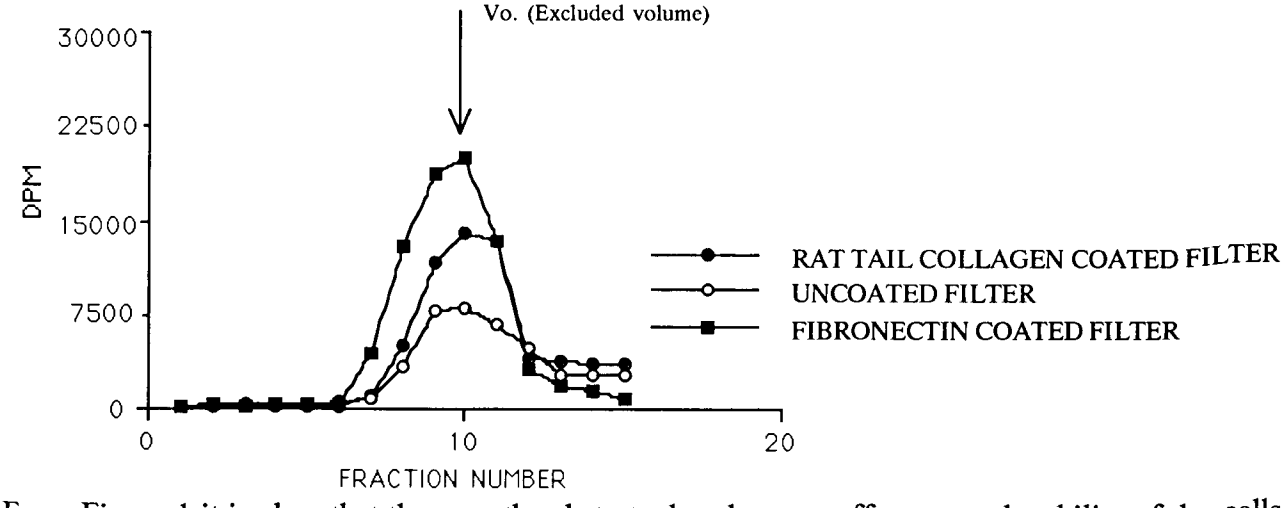
A number of cell lines have been established from human colonic adenocarcinoma tumours (Fogh et al 1977) and are used as in vitro models for gastrointestinal absorption and transport (Wilson et al 1990). It has been claimed that the HT29-18N2 clone, which represents a highly differentiated species produced by treatment of HT29 cells displaying absorptive properties with sodium butyrate (Huet et al 1987; Phillips et al 1988), secretes mucus. If this is so, then the validity and potential of the model are increased since the effects of the mucus barrier on absorption can be investigated in vitro.

The cells were grown on suspended nitrocellulose filters allowing access of media to both apical and basolateral surfaces. Filters were coated with various extracellular matrix components to determine their effects upon mucin secretion. 3H-glucosamine, which is known to be incorporated in the glycoprotein carbohydrate side chain, was added to the apical compartment of the filter at a concentration of 4μCi / ml for a period of 24 hours. The labelled medium was removed and the mucus glycoprotein separated by gel chromatography. Incorporated label was detected by scintillation counting of excluded fractions eluted from a Sepharose CL4B gel filtration column. In an attempt to confirm that any secretion was indeed mucus glycoprotein, periodic acid Schiff (PAS), alcian blue and a combination of alcian blue and PAS stains were performed on cell sections and enzymatic digests of material excluded from the gel chromatography.

FIGURE 1. RADIOACTIVITY IN FRACTIONS FROM GEL CHROMATOGRAPHY



From Figure 1 it is clear that the growth substrate does have an effect upon the ability of the cells to secrete a high molecular weight species with fibronectin producing the greatest yield. Results obtained from light microscopy using the PAS and alcian blue staining show columnar cells with granules staining positively for glycoprotein and evidence of an overlying mucus layer. However, no structures with the morphological features of goblet cells could be found. It is therefore concluded that the HT29-18N2 cell line does produce mucus but that this is secreted from granules within cells which appear columnar in nature.

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Fogh,J., Fogh, J.M., Orfeo,T. (1977). J.Natl.Cancer Inst. 59: 221-26
Huet,C. et al (1987) J.Cell Biol. 105: 345-357
Phillips,T.E. et al (1988) Gastroenterology 94: 1390-1403
Wilson,G. et al (1990) J.Controlled Release 11: 25:40