APPLICATION OF PRIMARY RAT COLON CELLS FROM BASAL AND SURFACE CRYPT-SECTIONS FOR PROLIFERATION AND GENOTOXICITY STUDIES

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Increased colon cell proliferation, especially in the basal crypt-sections, and genotoxicity, (leading to mutations in tumour oncogenes and suppressor genes,) are two parameters which contribute to the early steps of colon carcinogenesis. In order to study the impact of nutritional factors on these parameters, our efforts have been focused on establishing an isolation technique to obtain rat colon cells in different stages of differentiation. Rat colon cells were isolated stepwise from the villus tip to the basal crypt using a modification of the method of Schulman et. al., (Am.J.Physiol. 266 (Cell Physiol. 35): C729 - C740, 1994). These fractionated cells were then employed to study endogenous levels of DNA-damage, oxidized DNA-bases and proliferation rates. Additionally, the potential protective effects of short chain fatty acids (SCFA) on *in vitro* proliferation were examined.

We found that cell yield increased from fraction 1 to 6 and total cell yield of all fractions was 61 ± 25 millions. Viability, determined by trypan blue exclusion, was between 70 and 86%. The basic rate of cells with intact DNA from fraction 2,4 and 6, as measured by single cell microgelelectrophoresis (comet assay) was 35%, 47% and 53%, respectively. The degree of endogenous oxidative DNA-damage, visualized by treatment with endonuclease III, was increased by about 8% in fraction 2, 10% in fraction 4 and 26% in fraction 6. The proliferation rate evaluated by bromodeoxyuridine-incorporation and detection of proliferation nuclear antigen (both detected immunohistochemically) was not different in fraction 3 and 6 following 1 hour 45 minutes or 2 hours 45 minutes incubation. Furthermore, treatments by butyrate or acetate (6.25 mM) did not alter this parameter.

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ABSTRACTS

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