

## CHARACTERISATION OF AN ORGANIC ANION TRANSPORT MECHANISM IN THE HUMAN COLON CARCINOMA CELL LINE HT29 CLONE 19A

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HT-29 is a commonly used human intestinal tumor cell line to study various aspects of the impact of nutritional components and metabolites on processes of colon carcinogenesis. Clone 19 has been subjected to differentiation by treatment with butyrate. In order to use this all clone for studies aimed at determining the effects of nutritional factors, it is necessary to characterize the cells' physiological functions. We have measured Fluo-3 efflux in cultured HT29 cl. 19A cells grown on cover slips to investigate a recently identified probenecid sensitive organic anion transporter in these human colon carcinoma cells (Pflügers Arch. 429: R56, 1995). Cells were loaded with Fluo-3 upon incubation with Fluo-3/AM and 2 mM probenecid. Intracellular Fluo-3 fluorescence of a group of 6-12 cells was measured at 37°C using epifluorescence microscopy and a photomultiplier. The decrease in intracellular fluorescence after 5 min incubation was calculated as the percentage of the total cellular fluorescence intensity at the start of each experiment. Fluo-3 efflux was significantly reduced in the presence of 2 mM probenecid ( $6.2 \pm 3.7\%$  versus  $22.8 \pm 2.2\%$ ,  $n=5-6$ ,  $P<0.01$ ). Fluo-3 efflux also tends to be lowered at 25°C ( $8.2 \pm 1.3$  versus  $15.5 \pm 3.7\%$ ,  $n=5-6$ ,  $P<0.06$ ), but was not altered in the presence of 10 mM p-aminohippurate ( $18.0 \pm 1.0\%$ ,  $n=4$ ), 10 mM pyruvate ( $22.0 \pm 9.5\%$ ,  $n=3$ ), 10 mM  $\alpha$ -ketoglutarate ( $16.0 \pm 1.5\%$ ,  $n=4$ ), or 10 mM sulfate ( $14.5 \pm 0.5\%$ ,  $n=2$ ). We therefore conclude that HT29 cl. 19A cells express a probenecid sensitive anion transport mechanism that is not stimulated after addition of p-aminohippurate, pyruvate,  $\alpha$ -ketoglutarate or sulfate to the trans side.

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**ABSTRACTS**