IMMUNOLOCALIZATION OF CYTOCHROME P-450 ISOENZYMES IN HUMAN EPITHELIAL CELLS

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Cultured keratinocytes obtained from human hair follicles might be a useful tool to study mutagenicity in human epithelial cells. Human hair follicles possess a cytochrome P-450 dependent enzyme system which is capable to metabolize xenobiotics. The preservation of this enzyme in vitro is important for the application of hair follicle cell cultures in genotoxicity studies especially for promutagens and procarcinogens.
We studied the immunolocalization of cytochrome P-450 using monoclonal antibodies (K03 and K07) raised against two isoenzymes. The antigens were present in freshly plucked hair follicles, fibroblasts and the cell line SVK_{14}. In the cultured keratinocytes no staining was observed by the antibodies. Since the cell line SVK_{14} shows a medium dependent response on the antibodies, the absence of cytochrome P-450 in the hair follicle keratinocytes is ascribed to the culture conditions. Further studies on the relation between culture media and maintenance of cytochrome P-450 is required.

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A TWO-WEEK FEEDING STUDY OF BHA: EFFECT ON CELL KINETIC PARAMETERS IN THE RAT GASTRO-INTESTINAL TRACT.

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The synthetic food antioxidant 2(3)-tert-butyl-4-hydroxyanisole (BHA) is carcinogenic in the forestomach of rats, hamsters and probably mice. Sequential changes are dose-dependent and involve lesions, hyperplasia, papillomas and carcinomas, the development of which is accompanied by an increase in forestomach labelling index (L.I.). In the present study, subcellular markers of cell kinetics were assessed in the rat gastro-intestinal tract after short-term consumption of BHA.
Groups of five male Wistar rats (306±17g) were fed a diet containing 2% BHA or basal diet (control group) for two weeks. Subsequently, rats were injected i.p. with 25 mg/kg 5-bromodeoxyuridine (BrdU), a thymidine analogue, and killed after four hours. The gastro-intestinal tract was removed, opened longitudinally, cleaned and fixed in 70% ethanol. After pepsin digestion of random samples of the fixed tissues, labelled cell nuclei were visualized by means of a monoclonal anti-BrdU antibody technique. Cell kinetic parameters were determined by bivariate BrdU/DNA analysis using flow cytometry.
Forestomach L.I. and potential doubling time (Tpot) in random samples were 10.0±3.4% and 2.7±0.8 days for the control group and 20.7±3.9% and 1.2±0.2 days for the group fed 2% BHA respectively (mean ± SD; $p<0.001$). Mean transit time through the S-phase was not altered. Glandular stomach, ileum, caecum and colon were not affected.
Thus, we confirm proliferative effects of BHA on rat forestomach as indicated by an increase in L.I. and additionally report a decrease in Tpot following short-term dietary BHA administration.

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NANOMOLAR CONCENTRATIONS OF Cd^{2+} INHIBIT Ca^{2+} TRANSPORT SYSTEMS IN PLASMA MEMBRANES AND INTRACELLULAR Ca^{2+} STORES.

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Exposure of fish to cadmium (Cd) in the water causes a spectrum of toxic effects that is well documented. The mechanisms of Cd-toxicity, however, are largely unknown. A transient hypocalcemia is observed in fish the first days after Cd-exposure, which is indicative of a disturbed Ca^{2+} homeostasis. For freshwater trout it was demonstrated (1) that branchial Ca^{2+}-influx (which is a transcellular event depending on basolateral plasma membrane Ca^{2+} ATPase activity) is extremely sensitive to inhibition by Cd^{2+}. Experiments with isolated basolateral membrane (BLM) vesicles provided direct evidence that the Ca^{2+} pump in the BLM of gills is strongly inhibited by Cd^{2+} (IC_{50} = 3 nM at 0,25 Ca^{2+})
Not only in fish gills (trout, tilapia) but also in intestine and kidney from landvertebrates (rat, chicken) Cd was reported to inhibit transepithelial Ca^{2+} transport. Proceeding from the extreme sensitivity of the branchial Ca^{2+} pump we predicted that a high affinity of the Ca^{2+} pump for Cd^{2+} would be a general phenomenon and that the inhibition of the Ca^{2+} pump may be the key event in Cd-toxicity. In this study we tested the effect of Cd^{2+} on active Ca^{2+} transport in BLM's isolated from rat duodenum and rat kidney cortex. Using permeabilized duodenal cells we were able to study the effect of Cd^{2+} on ATP-dependent Ca^{2+} transport in intracellular stores. A kinetic analysis of the Cd^{2+} inhibition was undertaken to evaluate the mechanism of inhibition on the molecular level.

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MUTAGENIC ACTION OF SOME ISOCYANATES AND THEIR AMINE ANALOGUES TO SALMONELLA TYPHIMURIUM

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Organic isocyanates are highly reactive chemicals characterised by the general formula R(NCO)x. The diisocyanates are widely used for the industrial production of polyurethanes. Exposure to isocyanates is known to cause pulmonary and skin irritation as well as immunologic sensitization of the respiratory tract. In contrast to these well studied toxic effects, little is known about the mutagenic and possible carcinogenic effects of the isocyanates.
We present a study of the mutagenic action to Salmonella typhimurium of three isocyanates extensively used in polyurethane industry: toluene diisocyanate (TDI), 4,4'- diphenylmethane diisocyanate (MDI) and hexamethylene diisocyanate (HDI). In addition, the closely related tolylisocyanate was also studied. Isocyanates easily form amines in a reaction with water. Therefore the amine analogues (TDA, MDA, HDA and toluidine) were incorporated in the Ames-tests.
The mutagenicity testing was carried out with the plate incorporation assay as described by Ames et al. (1). The tests were performed with S. typh. strains TA 100, TA 1535, TA 98 and TA 1538 both with and without metabolic activation (S9-mix containing rat liver homogenate (9000 g)).
The isocyanates, particularly HDI, showed a large toxic effect on the Salmonella bacteria.
Mutagenicity was observed with TDI, TDA, MDI and MDA in TA 100 and TA 98 with S9-mix. In both cases the amine was more mutagenic than the analogous isocyanate. This finding suggest that the mutagenic effect of isocyanates can be attributed to reactive metabolites of the amines formed during hydrolysis of isocyanates.

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