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ONCOGENE EXPRESSION IN HUMAN BLADDER EPITHELIAL CELL LINES:

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A series of human bladder epithelial cell lines derived from both normal and malignant tissue has been investigated. Levels of cellular oncogene expression in a mortal, non-tumorigenic line of normal origin (Hu1752) were compared with the levels found in two immortalised cell lines derived from normal tissue (HCV29 and Hu609) and in an immortal, tumorigenic, bladder carcinoma cell line (T24). Hu609 and T24 overexpressed c-myc 20 fold while HCV29 and T24 overexpressed c-sis 20 fold.
The structure of the c-myc gene in Hu609 and T24 was normal at the level of Southern blotting and the stability of the c-myc RNA was unaltered, suggesting that 20 fold overtranscription of the normal c-myc gene occurs in Hu609 and T24. We are presently producing a recombinant plasmid in which the chloramphenicol acetyl transferase gene is placed under the control of a normal c-myc promoter. This construct will be used to investigate the enhanced c-myc transcription rate.

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FORMATION OF AN UNSTABLE IQ-DNA ADDUCT.

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The binding of the carcinogen, 2-amino-3-methylimidazo(4,5-f)quinolin (IQ), to DNA was investigated using an in vitro microsomal incubation system, HPLC separation techniques and standard DNA isolation methods. Using ^{14}C-guanine labelled DNA 9μCi/μmol DNA-guanine) and ^{3}H-IQ (180μCi/μmol), we were able to identify an unstable adduct that is released from IQ-adducted DNA within 2 hours from its formation. The general Danish population is exposed to IQ from fried meat. The identification of unstable IQ-DNA adducts may be useful for designing techniques to monitor the genotoxic exposure of individuals to IQ from fried meat.

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FREEZE FRACTURE STUDY OF PLASMA MEMBRANE OF DAUNORUBICIN SENSITIVE AND RESISTANT EHRLICH ASCITES TUMOR CELLS.

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The plasma membrane is considered to play a major role in the development and circumvention of multidrug resistance and is possibly also a target for anthracycline drug effect.
Freeze fracture replicas were obtained from unfixed sensitive (EHR2) and daunorubicin resistant (EHR2/DNR+) cells without cryoprotection. A significant ($p<0.001$) increase was found in the number and mean size of P (protoplasmic) face intramembraneous particles (IMP) in EHR2/DNR+ compared with EHR2. 3-dimensional histogram plots of small and large IMP axes indicate the presence of a subpopulation of large (approximately 10 x 12 nm) IMP in EHR2/DNR+ not found in EHR2. Incubation with 5μM daunorubicin for 30 min. at 37°C did not affect IMP distribution in either subline. However, verapamil (20μM for 30 min. at 37°C), caused IMP aggregation in EHR2/DNR+, and, to a lesser extent, also in EHR2.
Conclusion: 1) The results are consistent with the concept of the emergence of a high molecular weight transmembrane protein in resistant cells such as P-170. 2) Verapamil, but not daunorubicin itself affects plasma membrane structure.

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LEUCOCYTE-ASSOCIATED PLASMA PROTEINS, MOLECULAR WEIGHTS AND IN VITRO BINDING TO LEUCOCYTES AND HL-60 CELLS.

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Six plasma proteins isolated from sonicates of thoroughly washed leucocytes by crossed immunoelectrophoresis, were studied by PAGE. The MW's of prealbumin, albumin and transferrin were the same as for their normal serum counterparts, but orosomucoid had higher, and a_1-antitrypsin and haptoglobin much lower MW's than normal, indicating molecular complexes and broken down proteins.In vitro incubation of mononuclear leucocytes, granulocytes and HL-60 cells with radiolabelled human serum proteins demonstrated that heat-denatured albumin, but not native albumin, was taken up and broken down by all three cell types. Transferrin was also taken up by all cell types,whereas orosomucoid and haptoglobin bound to mononuclear leucocytes and granulocytes,but not to HL-60 cells. a_1-antitrypsin bound to one out of two HL-60 cell lines and to leucocytes when not damaged by the radiolabelling.

The studies suggest that the association of plasma proteins with leucocytes isspecific and differsamong the individual proteins, three of which may be reacting with cellular enzymes.