and drug administrations. It was concluded that (1) metabolic imbalnce of ornithine was markedly changed by polyamine depletion to leukaemia cells and (2) combined therapy enhanced the cytotoxicity of cyclophosphamide and increased the life span of tumour bearing animals by 200 to 300 per cent.

MITOCHONDRIA AS INTRACELLULAR TARGETS FOR ANTICANCER THERAPY

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The presence and expression of DNA within mitochondria is now well established. In a series of studies with various tumour model systems, it has been shown that inhibition of mitochondrial gene expression leads to cell proliferation arrest and in some cases even to tumour eradication. Tetracyclines, which specifically inhibit mitochondrial protein synthesis exert these effects. We consider depleted energy generation capacity to be the most likely explanation. Also MGBG, an inhibitor of polyamine biosynthesis, preferentially impairs mitochondrial biosynthetic processes. The presence of two genetic systems in all tumour cells raises the question whether or not the mitochondrial system is also a target in other treatments primarily designed to interfere with the nucleocytoplasmic system. For doxorubicin and cytosine-arabinoside, effects on mitochondrial biogenesis and function have been observed and the findings are under further investigation.

HERPES VIRUS SPECIFIED EARLY PROTEINS INDUCE CELLULAR DNA SYNTHESIS IN VIRUS INFECTED CERVICAL CANCER CELLS

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The mode of DNA synthesis in virus infected HSV-2 permissive and non-permissive cervical cancer cells was studied. The flow cytometric detection of BrdU in newly synthesized DNA was achieved using a monoclonal antibody. PFA was used to differentiate between cellular and viral DNA synthesis. In the permissive CaSki cells an exponential increase of both DNA-synthesis and amounts of infectious virus was seen. In the non-permissive C-33A cells, a comparable increase of DNA synthesis was seen 6 hr after infection, but not later. The inhibition of viral DNA synthesis by PFA was able to inhibit the virus induced DNA synthesis in the CaSki but not in the C-33A cells. In the CaSki cells the exponentially increasing DNA synthesis corresponded to the virus replication. In the C-33A cells a transient induction of DNA synthesis was noted. This is likely to represent virus induced cellular DNA synthesis. In the CaSki cells, HSV-specified major DNA-binding protein (ICSP 11/12) was seen in the nucleus, whereas in the C-33A cells the protein was located both in the cytoplasm and the nucleus. Early viral proteins are expressed also in the PFA treated cells and show (ICSP 11/12) affinity to DNA. Whether the early viral proteins mediate the virus induced increase in cellular DNA synthesis in the non-permissive cancer cells has been evaluated.

THE E3/19K PROTEIN OF ADENOVIRUS TYPE 2 BLOCKS CELL SURFACE EXPRESSION OF HLA CLASS ANTIGENS AND INTERFERES WITH THE IMMUNE RESPONSE

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The adenovirus type 2 encoded protein E3/19K binds to human HLA class I antigens. The formation of the HLA-E3/19K complex prevents the HLA antigens from being correctly processed by inhibiting their terminal glycosylation and cell surface expression. This reduced level of antigens influence the cytotoxic T cell response. Also the murine H-2 K^d antigen binds to the viral protein whereas the allelic K^k antigen does not. Hybrid genes between the K^d and K^k alleles were constructed and have allowed us to map the 1 and 2 domains of the class I antigen to be the essential structures involved in the complex formation. Interestingly, these domains are also crucial for T cell recognition.

ALKYLATING AGENT-INDUCED MUTAGENESIS AND ACTIVATION OF THE Ha-ras ONCOGENE

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Activation of the Ha-ras oncogene in rat and human cells is associated with a point mutation at a particular position, usually the 12th codon of the first exon. Although it has been postulated that the point mutation (G→A) observed in rat mammary tumours induced by the methylating carcinogen N-nitrosomethylurea is the result of misreplication of DNA containing the premutagenic lesion O^6-methylguanine, neither this nor any other relationship between DNA damage and oncogene mutagenesis/activation have been examined directly. Furthermore, since it has been suggested that the pattern of oncogene activation may be related to preceeding carcinogen-induced DNA damage and, hence, be carcinogen-specific, examination of the pattern of mutagenesis and activation induced in the human Ha-ras oncogene and its relationship to particular carcinogen-DNA adducts would be of particular interest in studies of the etiology of human cancer. Our current studies have involved research on DNA modification, mutagenesis and activation in the human Ha-ras oncogene (involving the construction in vitro of alkylated forms of the protooncogene, transfection into procaryotic or DNA sequqence modifications).

MODIFICATION OF THE INTRACELLULAR pH OCCURS DURING THE DIFFERENTIATION OF LEUKAEMIC CELL LINES (HL-60 AND U937) TOWARDS MONOCYTE LIKE CELLS

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We have measured the intracellular pH (pHi) during the monocytic differentiation of HL-60 cells induced by human recombinant interferon gamma (rHu-IFN-γ, RU42369), and of U937 cells induced by retinoic acid (RA).

pHi was monitored either by the fluorescence of intracellularly-trapped bis-carboxyethylcarboxyfluorescien, or by the distribution of [14]C benzoic acid. In both cases there is an increase in the pHi from 7.00 ± 0.03 to 7.13 ± 0.01 for rHu-IFN-γ treated cells, and from 7.02 ± 0.02 to 7.23 ± 0.03 for RA treated U937 cells.

In both cell lines the pHi is regulated by two mechanisms: a Na+/H+ exchange system and a Na+ dependent HCO_3^-/Cl^- exchange system which both catalyze an influx of [22]Na^+. Their pharmacological and biochemical properties have been defined. During the differentiation process, the activity of the Na+/H+ exchange system is increased at all the pHi values comprised between 6.20 and 7.60. The activation of this system is not a rapid phenomenon as observed with growth factors on quiescent cells. No activation could be detected during the first three hours of culture with the drug. The maximal effect is obtained two days after rHu-IFN-γ addition and three days after RA addition.

EVALUATION OF THE REACTIVE PRINCIPLES RESPONSIBLE FOR GENOTOXICITY AS A PREREQUISITE FOR CARCINOGENIC EXPOSURE MONITORING OF HALOGENATED ETHYLENES AND BUTADIENE

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For halogenated ethylenes and other alkenes, several reactive metabolites could be relevant for exposure monitoring. For vinyl chloride (VC) and vinyl bromide (VBr) the detection of 7-N-(2-oxoethyl)-guanine (a reaction product of the corresponding epoxide and guanidine) in liver DNA of rats after exposure of the animals to VC or VBr supported the central role of the epoxides as carcinogenic principle in metabolism of these compounds. A possible role of the reactive VC-metabolite chloroacetaldehyde (CAA) in formation of DNA-adducts and in genotoxicity of VC could be excluded on the basis of experiments with bischloroethylether, a CAA forming agent. Three different epoxides, epoxybutene (EB), epoxybutanediol and diepoxybutane have been suggested as reactive metabolic intermediates in butadiene metabolism. After exposure of mice to butadiene, 7-(1-hydroxy-3-buten-2-yl)guanine (a product of reaction of EB with guanine) could be identified in liver DNA of mice. This supports a central role of EB in BD induced carcinogenesis.

TRANSFORMING GROWTH FACTOR-BETA REGULATES THE PROTEOLYTIC ACTIVITY OF CULTURED NORMAL AND MALIGNANT CELLS

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Cultured embryonic fibroblasts (WI-38, CCL-137) and a fibrosarcoma cell line