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

A.Ladoux(1), C.Frelin(2), C.Damais(3), I.Krawice(1) and J.P.Abita(1)

(1)Unité INSERM 204, Hôpital St. Louis 75475, Paris Cedex 10, France; (2)Centre de Biochemie du CNRS, Parc Valrose, 06034 Nice Cedex, France; (3)Roussel Uclaf Research Laboratories, III Route de Noisy, 92320 Romainville, France.

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

R.J.Laib and R.Kreiling

Institut für Arbeitsphysiologie, Abteilung für Toxikologie, Dortmund, F.R.G.

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

Marikki Laiho, Olli Saksela and Jorma Keski-Oja

Department of Virology, University of Helsinki, Finland

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