SEMINAR PROGRAMME

Host Factors in Human Carcinogenesis

(Organized and chaired by H. BARTSCH).

Detection of Genotoxic Exposure

(Organized by H. AUTRUP and chaired by L. DRAGSTED).

Sequential Changes During Neoplastic Cell Transformation

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Leukocyte Adherence Inhibition Techniques in Cancer Detection

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(Organized and chaired by A. VAHERI)

Host-Factors in Human Carcinogenesis

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Host-factors that are recognized to play a role in human carcinogenesis are generally defined as molecular, metabolic and biochemical or genetic parameters which condition or predispose the human body to be more prone or resistant to the onset of malignancy by carcinogenic agents. Host-factors can be either inherited or acquired, and can operate at the systemic level or at the target cell level. The identification of such predisposing conditions together with hitherto unknown causative factors in the origin of human cancer is a major task. It is evident that conventional approaches in cancer epidemiology have provided valuable information, but they have serious limitations to identify the remaining specific causative factors in human cancer, in particular if cancer is the result from multifactorial origin. Also such classical epidemiological studies are largely retrospective rather than predictive and, unless very large numbers of individuals are studied, are not highly sensitive. On the other hand, animal bioassays and short-term tests are extremely sensitive and useful for detecting potential carcinogens, but today there is a limitation how such data can be extrapolated to humans. Progress in understanding cancer causation probably will come from two areas: an increased knowledge on the mechanism of carcinogenesis on the molecular and cellular level; and secondly from a newly arising approach, the so-called biochemical and molecular cancer epidemiology. Such studies use a variety of sensitive and specific procedures that have become available to assess biochemical and molecular parameters in man, which are related to exposure and to genetic and acquired host-susceptibility factors. This integration of laboratory measurements with more classical epidemiological studies should provide potential tools for cancer prevention prior to the onset of clinically evident cancer. Several examples of this new type of approach were presented at this Seminar.

F.F. Kadlubar (National Center for Toxicological Research, Jefferson, AR, USA) characterized some enzyme reactions that convert carcinogenic aromatic amines into reactive intermediates, with particular emphasis on those operating in human tissues.

Benzidine, a human and dog urinary bladder carcinogen, is known to be activated to DNA-bound products by peroxidative metabolism. Previous studies have shown that both one-electron and two-electron (diimine) oxidation products are formed as intermediates. The reactivity of synthetic benzidine diimine with DNA *in vitro* was examined and compared with the adducts formed during peroxidase catalysis. The diimine derived adduct was characterized as N-(deoxyguanosin-8-yl)-benzidine. Incubation of benzidine with H_2O_2/horseradish peroxidase or arachidonate/prostaglandin H synthase resulted in the formation of the same DNA-bound derivative. However, a second major adduct was also observed, and spectral analyses were consistent with C8-substitution of deoxyguanosine and ring-substitution of benzidine. Preliminary studies with dogs given benzidine orally indicated the same DNA adduct profile is present in the urinary epithelium. These data implicate benzidine

diimine as an important electrophilic intermediate in the peroxidative metabolism of benzidine in addition to other electrophilic intermediates.

The relationship between slow/fast acetylator phenotype in humans and metabolic activation of aromatic amines was also discussed. The genetic polymorphism in the rate of N-acetylation of drugs in humans is well known, and has been related to differential susceptibility to drug toxicity. Carcinogenic aromatic amines, such as 2-aminofluorene (AF), also undergo N-acetylation and slow acetylators have been suggested to have an increased risk to urinary bladder cancer. Acetyltransferases have also been shown to serve as a final metabolic activation step in the conversion of hydroxamic acids and N-hydroxy arylamines in DNA-bound adducts. In a recent study, the enzymatic activity of thirty-five human liver cytosols (obtained surgically from organ donors) for N-acetylase, N-,O-acyltransferase, and the acetyl CoA-dependent binding of N-hydroxy-AF to DNA was examined. The results indicated that about two-thirds of the human liver samples were of the slow acetylator phenotype; and the same individuals also exhibited a high rate of AF N-acetylation. Acetyl CoA-dependent activation of N-hydroxy-AF was detected in nearly all of the samples and was significantly higher in the fast acetylator group. These data suggest that fast acetylator individuals could be at higher risk to aromatic amine-induced cancer in those tissues containing appreciable levels of N-hydroxy arylamine O-acetylase, which was detected in human colon tissues as well. Thus it may be possible to relate an individual metabolic phenotype with a characteristic sensitivity to drugs or to carcinogens, and classify humans in terms of their rate of drug metabolism, propensity of development of xenobiotic toxicity and hyper-susceptibility to certain chemical carcinogens.

The contribution of M. Roberfroid (Unité de Biochimie Toxicologique et Cancérologique, Université Catholique de Louvain, Bruxelles, Belgium) was related to one of the major cancers in the western world, cancer of the large bowel, where specific causes have not yet been identified with certainty; therefore any new insight in the mechanism of this disease, probably of multifactorial origin, would provide new opportunities to predict cancer risk on an individual basis and thus offer new venues for cancer prevention. The increasing knowledge that in the multi-stage carcinogenesis process the transitions between different stages can be enhanced and inhibited by different types of agents, have led to investigations whether certain bile acids may be of importance in cancer etiology. Indeed there is growing evidence implicating bile acids (BA) in colorectal carcinogenesis: i) the presence of BA binding sites in cancerous colonic mucosa has been observed, ii) BA cause dysplastic changes in the colonic mucosa, iii) BA are co-carcinogenic in the rat colon and iv) co-mutagenic in the Salmonella mutagenesis assay, v) there is a suggested correlation between colorectal cancer in humans and the mean faecal BA concentration. But as shown recently neither lithocholic acid nor deoxycholic acid nor their sum was a discriminant, but the ratio lithocholic to deoxycholic acid was a good parameter which was related to adenoma size. One of the major challenges in the field of biochemical and molecular epidemiology is to identify individuals who were exposed to such 'promoting' agents, and more so to identify those who are highly susceptible to their effect.

A. Decarli (National Cancer Institute, Milan, Italy) presented preliminary results from an ongoing case control study to investigate the relationship between breast cancer and vitamin A. This study includes 200 cases of breast cancer and 200 hospital controls. Serum levels of retinol, b-carotene, vitamins B_2, C, E, cholesterol and some other components have been evaluated by interviews on dietary habits both in the cases and controls. The final results are being evaluated after stratification for the known or suspected risk factors for breast cancer.

H. Bartsch (IARC, Lyon, France) reported on collaborative studies with M. Crespi (Regina Elena Institute, Rome, Italy) to relate the intragastric formation of N-nitroso compounds (NOC) in humans to the induction of upper gastrointestinal cancer. It has been hypothesized that subjects with an achlorhydric stomach have an increased intragastric formation of NOC due to a large number of bacteria in their stomach that convert NO_3^- to NO_2^- and catalyse nitrosation. To test this hypothesis, patients with chronic atrophic gastritis (CAG), pernicious anaemia and who have undergone gastrectomy are being analysed for the levels of nitrosated amino acids in urine after application of the N-nitrosoproline (NPRO) test. NPRO levels in CAG patients were found to depend on gastric pH (max. yield at pH 1.7 with large inter-individual variations (0 to 120 mg/day) of NOC excreted). CAG patients, as compared to controls, excreted no excess of NPRO, but their gastric juice contained higher NO_2^- levels. However, recently 25 out of 35 bacterial strains (isolated from human sources) were found that exhibited nitrosation activity at pH 7 in vitro. The formation of N-nitrosomorpholine followed Michaelis-Menten kinetics and substrate specificity for several amines was found (proline being a poor substrate), suggesting a nitrosation catalysis by bacterial enzyme(s). These data clearly indicate that endogenous nitrosation does occur in the human stomach, but its relation to the induction of upper gastrointestinal cancer remains to be proven.

A.R. Lehmann (MRC Cell Mutation Unit, University of Sussex, UK) reviewed current knowledge on the molecular mechanism underlying cancer-prone genetic disorders with defects in DNA repair. A number of such disorders are associated with cellular defects in the ability to repair damaged DNA. In xeroderma pigmentosum there is a clear relationship between defective DNA repair, enhanced UV mutagenesis and an increased frequency of skin cancer. In Cockayne syndrome, ataxiatelangiectasia and in an immunodeficient individual (46BR), there is strong evidence for different defects in DNA repair, which give rise to cellular hypersensitivity to the lethal

effects of different mutagens. In these disorders, however, the relationship of the molecular and cellular defects to the clinical symptoms of the diseases are very complex. Attempts are now being made to clone the genes which are defective in these disorders, in order to gain a better understanding of their molecular bases. Using the increasingly powerful tools in DNA recombinant technology, it would be feasible to identify subjects with cancer pre-disposing genes in the general population, including those with the heterozygous carrier state. This will be yet another example where a new discipline, genetic (mutation) epidemiology, may start to operate more efficiently, based on the new powerful tools in molecular and cellular biology.

Detection of Genotoxic Exposure

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Detection of genotoxic exposure has developed to an important stage leading to two major directions of research. One of these is concerned with refinements of traditional methods for the testing of potentially genotoxic compounds. The other aims directly at the detection of human exposure to genotoxic compounds, mainly through measurement of DNA-adducts or DNA- repair products.

Epidemiological studies, linking environmental exposures with human cancer are still the only way to positively associate genotoxicants with disease in man. This approach, however, suffers from the lack of precise measurement of dose and, even more important, from a lack of ability to discern between the many potentially genotoxic exposures that exist in the human environment.

The tremendous advances in developing test systems to identify potentially genotoxic and/or carcinogenic compounds has brought a large number of substances into consideration as potential human genotoxicants.

J. Hradec (Oncological Institute, Prague, Czechoslovakia) gave an overview of the tRNA-acceptance assay, a short-term test that takes advantage of the observation that many carcinogens enhance the charging of initiator tRNA with methionine. A large number of compounds, especially nitrosamines and triazines have been tested, and a provisional validation of the test shows good ability to identify carcinogens from several chemical classes.

M. Červinca (Charles University, Hradec Kralove, Czechoslovakia) presented a microcinematographic method for increasing the amount of information gained from in vitro tests on cellular systems. With this method, individual fates of cells, e.g. toxicity, change in morphology, etc. may be followed closely. Such observations will contribute to the discrimination between different mechanisms of action of test substances that lead to the same change in endpoint at a populational level.

D. Slameňová (Slovak Academy of Sciences, Bratislava, Czechoslovakia) presented work on tests for DNA damage in human fibroblasts, compared with results obtained with the Chinese hamster V79 HGPRT gene mutation test. A striking correspondence between test results obtained with these two test systems was observed, albeit on a small quantity of test substances.

S. Parodi (University of Genova, Italy) gave an overview of his data-base that contains a compilation of results from several short-term tests and animal carcinogenicity tests. The single test results are transformed to potency values, i.e. dose to effect relations, and quantitative correlations between tests are calculated using these values for all compounds tested. The data-base approach provides two major advances compared to traditional use of short-term test results. First, the choice of tests for a predictive test battery can be founded on practical results. Generally, the more remote the end-points or mechanisms in two short term tests, the less their correlation and the greater the gain in information and in predictivity of the two tests in combination. Also, as correlation between carcinogenicity tests and some short-term tests is not favourable for certain groups of chemical substances, the choice of tests for a predictive battery can be made dependent on the chemical structure of the test compound. Second, though correlation of potencies between single short term tests and animal carcinogenicity tests are generally of the order of 0.4, a better quantitative risk assessment might be obtained through studies of this kind.

In parallel with test development, a much better understanding has been gained of the early effects that genotoxicants cause in the cell and important refinements of analytical techniques have made possible the measurement of such early effects in exposed humans.

K. Vähäkangas (University of Oulu, Finland) presented work on the detection of benzo(a)pyrene DNA-adducts in human tissues by synchronous fluorescence spectrophotometry (SFS). The sensitivity of this method is comparable to that of ultrasensitive immunoassays (USERIA) and ^{32}P-post-labelling techniques. As SFS is faster and much less laborious than the other techniques, it may be advantageous for use in combination with epidemiological studies. With SFS or USERIA it is possible to detect benzo(a)pyrene DNA-adducts in lymphocytes from coke-oven workers. The number of positives obtained with the USERIA assay was somewhat larger than that obtained with SFS.

F.F. Kadlubar (National Center for Toxicological Research, Jefferson, Arkansas, USA) described approaches to the detection of genotoxic exposure from aromatic amines. 4-aminobiphenyl was used as a model compound during development of analytical techniques in exposed animals. ^{32}-post-labelling in combination with HPLC was used to detect DNA-adducts. Surprisingly low levels of 4-aminobiphenyl DNA-adducts can be identified even in non-dosed animals. Furthermore, several unidentified "natural" adducts were found, and may represent quantitatively important background exposures.