Meeting Report

Conference on Advances in the Biology and Chemistry of N-Nitroso and Related Compounds

To celebrate its 25th anniversary, the Eppley Institute for Research in Cancer organized this conference on "Advances in the Biology and Chemistry of N-Nitroso and Related Compounds." The members of the organizing committee were the authors of this report. About 130 scientists from 15 countries attended. The opening and closing lectures, given by R. D. Adamson (National Cancer Institute, Bethesda, MD) and P. N. Magee (Fels Institute for Cancer Research, Philadelphia, PA), respectively, emphasized the importance of N-nitroso compounds [RN(NO)R', NNC] in cancer research. These compounds are versatile carcinogens which have served as important models for the development of new strategies for cancer prevention and therapy. The available evidence indicates that NNC not only provide excellent models for inducing tumors at specific sites and studying the mechanisms of carcinogenesis, but are also significant causes of cancer of the stomach, esophagus, nasopharynx, and oral cavity in humans.

Sessions of the conference covered chemistry and analysis, endogenous formation and dosimetry, metabolism, DNA alkylation and repair, mutagenesis and molecular biology, and biological effects.

Chemistry and Analysis

M. Wiessler (German Cancer Research Center, Heidelberg, FRG) summarized studies on the chemistry of α-acetoxy-nitrosamines and related derivatives. These compounds are important as stable models for the probable proximate carcinogens of nitrosamines, the α-hydroxy derivatives. The identification of glucuronide conjugates of α-hydroxy-nitrosamines in the urine of nitrosamine-treated animals demonstrates that the α-hydroxy intermediates are sufficiently stable for conjugation to occur. H. Ohshima (International Agency for Research on Cancer, Lyon, France) reported that urinary 3-methyladenine can be identified as a-diazoquinones, are produced by the nitrosation of phenols that occur in smoked fish and meat products. J. H. Hotchkiss (Cornell University, Ithaca, NY) described a method for analyzing nonvolatile nitrosamides. These are photolysed to give nitric oxide, which is then carried in a stream of helium into a Thermal Energy Analyzer (a nitric oxide detector normally used to determine nitrosamines). K. D. Brunnenmann (American Health Foundation, Valhalla, NY) described a modification of the Thermal Energy Analyzer for determining azoxyalkanes [RN(O):NR']. These carcinogens can readily arise by chemical oxidation of primary amines.

A new nitrosamine, 2-ethylhexyl-4-(N-methyl-N-nitrosamino)benzoate, was detected by H. J. Chou (Food and Drug Administration, Washington, DC) in sunscreen preparations. G. Eisenbrand (University of Kaiserslautern, Kaiserslautern, FRG) reported that previous assays had shown 10–270 ppb N-nitrosodiamethyamine in German cosmetics. Since secondary amines were banned from cosmetics in that country, only a few samples have tested positive for this nitrosamine, suggesting that elimination of NNC in commercial products is a realistic goal.

The rapid nitrosation of certain amidines, which are constituents of naturally occurring substances and commercial products, to yield dialkyl-nitrosamines was reported by R. N. Lepkyy (University of Missouri, Columbia, MO). S. S. Mirvish (Eppley Institute for Research in Cancer, Omaha, NE) studied the nitrosation of creatinine, a major food constituent which could yield NMU via the formation of two cyclic oximes. The first step leading to these oximes proceeds only very slowly. Kinetic studies by J. R. Leis (University of Santiago, Spain) indicated that indoles, thioproline, and simple amides are initially nitrosated at C, S, and O, respectively, and that the nitroso group then migrates intramolecularly to form NNC. This explains why these compounds are nitrosated more readily than expected.

Endogenous Formation and Dosimetry

H. Bartsch (International Agency for Research on Cancer, Lyon, France) presented an overview of endogenous nitrosamine formation in humans, as measured by the N-nitrosopropyl test developed in 1981. This test has been applied to populations in Japan and China with high incidences of stomach and esophageal cancer that may be caused by intragastric NNC formation. The nitrosopropyl test has generally indicated higher NNC formation in high risk subjects. However, individual levels are greatly affected by dietary modifiers and disease states. D. Shuker (International Agency for Research on Cancer, Lyon, France) reported that urinary 3-methyladenine can be used as a marker for DNA methylation by NNC in humans, since it does not normally occur in urine. A novel mechanism of in vivo NNC formation was reported by M. A. Marletta (University of Michigan, Ann Arbor, MI), who found that murine macrophages activated with lipopolysaccharides and other substances can synthesize nitrite and nitrate from the guanidine nitrogens of arginine, and that nitrosamines can be formed in this system. S. Leach (Bacterial Metabolism Research Laboratory, Salisbury, England) reported that denitrifying bacteria can nitrosate amines under anaerobic conditions in a reaction that may be catalyzed by nitrite reductase. R. C. Massey (Ministry of Agriculture, Fisheries and Food, Norwich, England) found that the level of apparent total NNC in the large intestine was higher in conventional than in germ-free rats treated with nitrate, reviving the view that NNC can be produced by intestinal bacteria.

Metabolism

W. Levin (Hoffmann-La Roche Inc., Nutley, NJ) and C. S. Yang (Rutgers University, New Brunswick, NJ) discussed their
studies on the identification of cytochrome P450j (P-450ac, P450IIIE1). This isozyme appears responsible for most of the metabolic activation of <1 mM concentrations of NDMA in rat hepatic microsomes and is induced by acetone, ethanol, isoni-
acid, 4-methylpyrazole, fasting and diabetes. Its activity is en-
hanced by cytochrome b5 and inhibited by specific monoclonal antibo-
dies. Cytochrome P-450j also metabolizes N-nitrosometh-
thylylamidine and N-nitrosodiethylenetriamine to varying extents. In the case of N-nitrosomethylbutylyamine, cytochrome P-450j is responsible for demethylation, whereas the phenobarbital-
inducible cytochrome P-450b catalyzes debutylation according to C. S. Yang. These workers and R. Hines (Eppley Institute for Research in Cancer, Omaha, NE) reported evidence that some inducers of cytochrome P-450j act by stabilizing its mRNA. P. Iversen (University of Nebraska Medical Center, Omaha, NE) presented data indicating that cytochrome P-450j activity in rat liver increases after partial hepatectomy. H. Ishizaki (Rutgers University, New Brunswick, NJ) studied the competitive inhibition of NDMA demethylation by alcohols and methylketones. A minimum Ki was obtained with com-
pounds containing 6 or 7 carbon atoms, suggesting that the hemoprotein has a hydrophobic binding site of about this size.

The metabolism by rat liver microsomes of N-nitrosomethyl-
ylamidine, an esophageal carcinogen, was reported by Mirv-
ish. Every carbon is hydroxylated, but hydroxylation at the 1
and ω-1 positions predominates. All hydroxylation activities were induced in the liver by phenobarbital, whereas only 3-
hydroxylation was induced by 3-methylcholanthrene. E. Richter (Walther-Straub-Institut, Munich, FRG) reported studies on the in vivo metabolism of the bladder carcinogen NDBA. First-
pass metabolism to 4-hydroxy-NDBA, a proximal carcinogen of NDBA, was rapid in lung and small intestine. Liver mainly produced 3-hydroxy-NDBA, which is not believed to be a proximal carcinogen of NDBA. G. W. Harrington (Temple University, Philadelphia, PA) reported that the liver showed an unusually high first-pass clearance of NDMA in the pig. H. M. Schuller (University of Tennessee, Memphis, TN) found that hamsters simultaneously exposed to 70% oxygen and treated with N-nitrosodiethylenetriamine developed neuroendocrine-cell tumors of the lung, perhaps in part because these cells proliferate at high oxygen levels. This procedure may provide a useful model for human oat-cell lung cancer. E. Frei (German Cancer Research Center, Heidelberg, FRG) reported the induction of spinal tumors in rats by methyl nitramine, a stable compound (unlike methylnitrosamine) produced by metabolic demethyla-
tion of dimethylnitramine.

DNA Alkylation

G. P. Margison (Paterson Institute for Cancer Research, Manchester, United Kingdom) cloned the O6-methylguanine transference gene from Escherichia coli, inserted it into a plas-
mid, and used this plasmid to construct a mammalian expres-
sion vector, which in turn was transferred into a variety of repair-deficient mammalian cells. By this means he investigated the effects of adduct repair after these cells were exposed to alkylating and chloroethylation agents. The results strongly support the importance of O6-alkylguanines in mutagenesis. L. Den Engelse (Netherlands Cancer Institute, Amsterdam, Neth-
erlands) reported that methylation of the phosphate backbone is somewhat stereoselective, but that both phosphotriester enan-
tomers disappeared from the liver at the same rate. The per-
sistence of these phosphotriesters may make them useful mon-
itors for human exposure. J. Bax (Netherlands Cancer Institute, Amsterdам, Netherlands) used immunocytocchemical methods to investigate the cell-specific formation and repair of O6-
methylguanine in tissues of hamsters and rats treated with BOP. O6-Methylguanine accumulated in DNA of pancreatic duct cells of hamsters, the target cells for BOP carcinogenesis in this species. D. Nagel (Eppley Institute for Research in Cancer, Omaha, NE) reported on studies in which O6-methylguanine was detected in liver and pancreas DNA of hamsters and rats treated with BOP; O6-(2-hydroxypropyl)guanine was also detected in liver DNA. B. Ludeke (University of Zurich, Zurich, Switzerland) described the preparation of rabbit antibodies to O6-(2-hydroxyethyl)deoxyguanosine and their use to detect DNA hydroxylethylation by several NNC, including N-nitroso-
2-hydroxyethylurea and N-nitrosodiethanolamine. Repair of O6-2-hydroxyethylguanine in the liver showed an unusually short half-life of 12 h. M. B. Kroeger-Koepeke (Frederick Cancer Institute, Frederick, MD) reported that 2-hydroxyethylation (but not methylation) of liver DNA by the liver carcinogen N-
nitrosomethyl-2-hydroxyethylamine was inhibited by 2,6-di-
chloro-4-nitrophenol, a suppressor of sulfation, suggesting that the nitrosamine sulfate ester was involved as a proximal carci-
ogen. The detection of 7-(2-cyanoethy1)guanine and O6-(2-cy-
anoethy1)guanine in liver, nasal mucosa, and esophageal DNA of rats treated with 3-(methyl nitrosamine)propionitrile (found in betel nuts) was reported by B. Prokopczyk (American Health Foundation, Valhalla, NY).

d Molecular Biology and Mutagenesis

S. Sukumar (Salk Institute, La Jolla, CA) reviewed evidence for the role of ras oncogene activation in mammary carcinogen-


esis by NMU. A single i.v. injection of NMU to pubescent rats specifically induced mammary carcinomas, 86% of which con-
tained the Ha-ras oncogene, activated in each case by a G-to-
transition in codon 12. This transition is the expected result of NMU treatment because this should produce O6-methyl-
guanine in the protooncogene. Phenotypic expression of the tumorigenic properties also requires proliferation of the mam-
mary cells during sexual maturation. K. Church (Eppley Insti-
tute for Research in Cancer, Omaha, NE) showed that NMU reacts at the N-7 position of guanine, with selectivity for oligo(dG) in G1 runs and additional selectivity for the central gua-
nine in G1 runs. This selectivity for runs of guanine can be overcome by linking the NMU to an intercalating methidium nucleus. R. K. Elespuru (Frederick Cancer Research Facility, Frederick, MD) and J. Guttenplan (New York University, New York, NY) described multiple-locus mutagenesis assays for characterizing proximal mutagens. The relative frequencies of mutations at various loci in E. coli strains were similar when common alkylating intermediates were produced from a variety of NNC. Both reports found that methylnitrosating NNC mainly produced GC-to-AT transitions, whereas higher homologues produced more GC-to- TA transversions. This approach was used, Guttenplan reported, to show that smokeless tobacco extracts behaved like methylnitrosating NNC. P. F. Swann (Middlesex School of Medicine, London, United Kingdom) used de-
tailed nuclear magnetic resonance studies to examine confor-
mational perturbations in duplex DNA structure caused by the replacement of guanine by O6-alkylguanine.


Biological Effects

S. S. Hecht (American Health Foundation, Valhalla, NY) reviewed recent studies on the biological properties and meta-
bolic activation of NNK and NNN, which occur in tobacco. Both nitrosamines induce esophageal tumors in rats. NNK, but not NNN, is also highly potent for tumor induction in rodent lung. The formation of O\(\text{6}\)-methylguanine in lung DNA appears important in this process. NNK also induces tumors of the exocrine pancreas in rats, supporting its role as a causative factor for pancreatic cancer in smokers. A cyclic N-7-C-8 adduct, formed by the addition of an oxobutyl group to guanine, was identified in liver DNA of rats treated with \(N\)-nitrosopyrrolidine. F. L. Chung (American Health Foundation, Valhalla, NY) reported that phenethyl isothiocyanate inhibited NNK-induced DNA methylation in rats, and lung tumor induction by NNK in rats and mice. P. Pour (Eppley Institute for Research in Cancer, Omaha, NE) reported that exogenous testosterone inhibited the induction of colorectal cancer in rats by BOP. Evidence was presented that testosterone acted during initiation by BOP and not during the subsequent promotion phase. M. P. Holsapple (Medical College of Virginia, Richmond, VA) described the suppression by NDMA of the antibody response in lymphocytes that were cultured with primary hepatocytes (required to activate the NDMA).

Conclusions

The results reported here are only a sample of those presented at this conference. They were selected to illustrate the advancing understanding of the mechanisms of NNC carcinogenesis, as well as the growing evidence for their significant involvement in human cancer etiology. These advances provide promising insights for developing strategies for cancer prevention.

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