[CANCER RESEARCH 42, 2099–2100, May 1982]
0008-5472/82/0042-0000$02.00

Meeting Report

Modified Nucleosides and Cancer

The first international workshop in this area was convened by Professor G. Nass of the German Central Laboratory for Studies of Mutagenicity.[1]

Results of studies of the modification of tRNA and DNA were presented. Of these, reports on the modifications of tRNA were the most numerous.

G. Dirheimer (Strasbourg, France) has counted 14 modified bases in tRNAs of cancers which are different from those of normal tissue counterparts. Interestingly, some of these are hypomodified and some are hypermodified; the most complex of modifications are generally undermodified. The complete synthesis of the Y-base of tRNA was presented by associates of S. Nishimura (Tokyo, Japan). The origin of the Q-base was presented by J. Katze (Knoxville, Tennessee). This is a fascinating modification inasmuch as it is not synthesized at the macromolecular level, but rather it is inserted by a specific enzyme. The enzyme was discovered by W. Farkas (Knoxville, Tennessee), who gave it the name guanine insertase. It inserts guanine or the Q-base into specific positions in tRNA, and quite remarkably it can insert the Q-base from the media of cells grown in tissue culture; thus, the base is a nutritional factor.

Nishimura and his group have shown that the Q-base modification of tRNA is low in tumor tissue and thus can serve as a substrate for the guanine insertase. This is an area of nucleic acid modification which needs very extensive study. One of the more fascinating aspects is that *in vitro* the enzyme requires no exogenous energy.

Early workers in the field of tRNA methylation have concluded from poorly designed experiments that methylation of tRNA has no function whatever. H. Kersten (Erlangen, Federal Republic of Germany) has nevertheless undertaken resolution of this difficult question. Essentially, she dissects the protein-synthesizing system by withholding one at a time the factors that are needed for complete synthesis. Then, by using methyl-deficient and methylated tRNAs, she can definitely state that the methyl group in the 23rd position of tRNA, which is ubiquitous in all tRNAs except the initiating tRNA, is essential for ribosomal binding. She is continuing these experiments.

There were several communications on the excretion of modified RNA nucleosides in the urine of tumor-bearing animals and cancer patients. G. Nass (Freiburg, Federal Republic of Germany) addressed the questions of the time course of the appearance of the modified nucleosides in the urine and also the amounts relative to tumor size. When mice are given injections of the carcinogen 3-methylcholanthrene, a tumor develops *in situ*. In the 16th week, there is a palpable 1-μl tumor under the ventral skin of mice, and death usually occurs around the 23rd week.

Modified nucleosides of 24-hr urine samples were determined from the initiation of the experiment until the demise of the animals. By the seventh week, when the tumor was not able to be diagnosed by palpation, excretion of the nucleosides was elevated. In the 16th week, the levels of the various nucleosides may be elevated as much as 2- to 4-fold above those of untreated controls.

While it has been demonstrated earlier that tRNA turnover is very high in tumor tissue, there was very spirited discussion whether the total amount of excretion products comes from the tumor mass itself. S. J. Kerr (Denver, Colo.) described some suggestive experiments which may bear on this question.

On incubation of cells in tissue culture with either 7-methylguanine or 1-methylguanine, normal breakdown products of tRNA, after 8 transfers, the cells were transformed and rendered oncogenic when tested in nude mice. The natural question is whether this may not occur in the whole animal as these products are leaving the site of the tumor toward the serum and eventually the urine. This area also needs intensive study.

More discriminating methodologies for the determination of other nucleosides were presented by C. Gehrke (Columbia, Mo.), who developed the most widely used quantitative determinations of these products. By a triple elution procedure, Gehrke observed several new degradation products of nucleic acids which promise to expand the sensitivity of these determinations. Incidentally, all of the participants at the meeting who are analyzing the products in the urine and body fluids paid tribute to Dr. Gehrke's meticulously described methods which they are all repeating successfully.

E. Borek (Denver, Colo.) reported on 2 heretofore unknown sources of BAIB[2] in the urine. It was observed that children whose crania are exposed to large doses of X-irradiation for extirpation of residual leukemia cells excrete high levels of BAIB, the origin of which then became obvious. It must come from DNA destruction as a result of X-irradiation and excision by the so-called S-O-S enzymes of the damaged DNA, resulting in accumulation of thymine in the cytoplasm which in turn is degraded into BAIB. There is only one other reference to this phenomenon. It came from a group at the Los Alamos Laboratories who analyzed the urine of subjects who were unwittingly exposed to a critical mass of uranium in an unfortunate accident. The individual who was demonstrating the assembly was killed, but the survivors were found to excrete BAIB in levels which were approximately proportional to the distance from the source of the energy. Extensive studies are needed to ascertain whether this excretion can be quantitated relative to the dose received and this might serve as a measure of radiation damage received from some catastrophic release of radiation. Borek and associates also observed that subjects who have been exposed to lead poisoning excrete high levels of BAIB. This is evidence of some DNA damage by the lead.

I. Clark (Piscataway, N. J.) reported on different patterns of excretion by rats with chemically induced hepatomas or neph-

[1] A workshop on "Modified Nucleosides and Cancer," sponsored by the Deutsche Forschungsgemeinschaft, was convened by Dr. Gisela Nass, Professor of Genetics, from September 28 to October 2, 1981, in Freiburg, Federal Republic of Germany. All communiques will be published *in extenso* in a special tissue of *The International Journal of Cancer Research and Clinical Oncology*.

Received December 21, 1981; accepted February 11, 1982.

[2] The abbreviation used is: BAIB, β-aminoisobutyric acid.

roblastomas. While the nucleosides excreted are the same, the levels of excretion are highly varied. His finding adumbrates well for the possibility of differential diagnoses, once enough information is accumulated. Measurement of diminished excretion levels of the nucleosides as an assessment of the effectiveness of chemotherapy is being used by F. Salvatore (Naples, Italy), G. Bjork and T. Rasmuson (Umea, Sweden), and G. Schöch (Hamburg, Federal Republic of Germany).

P. A. Jones (Los Angeles, Calif.) showed some striking effects of the incubation of mouse embryo fibroblasts in tissue culture with 5-azacytidine. This compound inhibits the methylation of DNA far in excess, probably due to conformational changes, of its stoichiometric equivalents in DNA. Cells with the attributes of muscle, irritability, etc., appear in some areas. Also, fat cells appear in the midst of the original fibroblasts. A number of other investigators have shown that the inhibition of DNA methylation by this compound produces a variety of different incipient differentiations.

Thus, the saga of the function of methyl groups in DNA is coming slowly to an end. It had been suggested soon after the discovery of methyl groups and methylating enzymes of DNA that the reaction serves as a sink for waste methyl groups. The work of Arber laid such an interpretation to rest.

It was also suggested by the late Jacques Monod that the methyl groups serve as punctuation for the end of certain genes. This could be rejected on the basis of very simple quantitative considerations. Bovine DNA contains twice as many methyl groups as does human DNA. This would indicate that the bovine genes are much shorter and, since the insulins produced by the 2 species are identical, this hypothesis could be rejected. The other possibility was that, in our advance to *Homo sapiens*, we have either forgotten or dispensed with punctuation.

The recent work with 5-azacytidine shows that the methyl groups in eukaryotes are not waste products; they are not punctuation, but rather they form some kind of shutters which expose or cover genes as needed for steps in differentiation.

Three novel approaches to the study of interactions of alkylating carcinogens with DNA were described. M. Rajewsky (Essen, Federal Republic of Germany) has developed high-affinity, very specific monoclonal antibodies to alkyl derivatives produced in DNA by alkylating carcinogens. These will provide a sensitive assay method for the quantitation of DNA damage and subsequent repair processes.

Ever since Sir Alexander Haddow discovered that alkylating carcinogens alkylate DNA, it had been tacitly assumed by many investigators in the field that the extent of the burden on DNA determines susceptibility to oncogenesis by the tissue. Such a conclusion was challenged by 2 investigators at the meeting.

P. Kleihues (Freiburg, Federal Republic of Germany) has conducted extensive experiments on the relationship of DNA alkylation by carcinogens and repair capacity of cells in a search for the source of the well-known organ and species specificity of tumor induction. He found a correlation between tumor incidence and initial extent of DNA alkylation for many organs, except for liver, which is extraordinarily resistant to chemical carcinogenesis. In the rat, tumor induction can be correlated with relative repair deficiencies for O^6-alkylguanine in target organs. However, this relationship does not extend across other species and strains of animals, where correlations of repair capacity and tumor induction are not good.

G. Neumann (Würzburg, Federal Republic of Germany) administered radioactive aminostilbenes which specifically induce tumors in the ear ducts. The binding to DNA by this carcinogen varies in tissues by a factor of 20. He measured the following parameters in a variety of tissues: total initial DNA binding; initial pattern of DNA adducts; persistence of adducts; and the accumulation of DNA binding after repeated doses. None of these parameters supports the hypothesis that the extent and persistence of DNA lesions determine the susceptibility of the tissue. Essentially, there was no difference in these parameters among the various tissues to which this carcinogen is innocuous. Thus, there is no unifying hypothesis for tissue and species specificity of alkylating carcinogens. Cancer researchers have "a darn long row to hoe."

Ernest Borek
University of Colorado School of Medicine
Denver, Colorado 80262