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Meeting Report

Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens[1]

In the context of research in the broad area of nutrition and cancer, a major new discovery was made during 1976, quite recently, when T. Sugimura (National Cancer Center, Tokyo, Japan) observed the unexpected presence of considerable mutagenicity at the surface of grilled or broiled fish or meat, using the usual procedures of cooking such foods. Isolation and identification of the mutagens showed that their chemical structure was that of a new class of chemicals, HCAs.[2] These powerful mutagens were also found active in other *in vitro* bioassay systems, providing evidence of their reactivity with the genetic apparatus. By now, an appreciable number of such compounds have been identified in cooked foods. Bioassays showed them to be potent carcinogens not only in the usual rodent systems but also in nonhuman primates. This research attracted worldwide interest, and many laboratories are now participating in a concerted, multidisciplinary effort to investigate the many facets bearing on the formation and mode of action of these newly discovered "environmental" carcinogens, with wide public exposure, mainly through p.o. intake.

Thus, an exciting scientific event, the Twenty-third International Symposium of the Princess Takamatsu Cancer Research Fund was held in Tokyo on November 10–12, 1992, under the heading "Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens." It included speakers from Australia, Japan, Norway, Sweden, Switzerland, United Kingdom, and United States, covering the current status of research in this new area. Application of rapid, contemporary methods led to the accumulation of relevant information on the significance of these chemicals in the human environment. This is expressed, in part, in the title of the Symposium "possible human carcinogens." In my opinion, this is remarkable, in view of the short life of this field in nutrition and health. The father of this field, Sugimura, was recognized with his Nakahara Memorial Lecture under the title "Past, present and future—Carcinogenic heterocyclic amines in cooked foods." While alluding to the historic aspects, the lecturer emphasized carcinogenicity data; the underlying mechanistic aspects, with emphasis on molecular interaction and consequences; and finally the key problem of the significance in human disease risk. Sugimura comments on multiple carcinogenic environmental elements, which, coupled with multiple cellular genetic changes as well as multiple steps in carcinogenesis, emphasize the resulting uncertainties in cancer risk assessments. He suggested, in a recent brief review (1), a pragmatic approach to cancer control, that does not involve "an excessive burden on the individual or community." Epidemiologic observations, discussed by M. Gerhardsson de Verdier (Karolinska Institute, Huddinge, Sweden), and Lang (Lawrence Livermore National Laboratory, Livermore, CA) provided support for an association between regular intake of well-done meats and gravies and risk of several types of cancer, including large bowel, urinary bladder, and pancreas.

S. Grivas (Swedish University of Agricultural Sciences, Uppsala, Sweden) reviewed practical methods of synthesis, and a number of HCAs are available commercially because of sizable demand. A key contributor with pioneering studies on the mechanisms of formation of HCAs, M. Jägerstad (Chemical Center, Lund, Sweden), demonstrated the important role of creatinine in the formation of the methylaminoimidazo moiety of the HCA molecules during Maillard reactions and provided new information on which elements are critical. The right proportions of amino acids, creatinine, and simple sugars are important. Enhancement was seen with iron and unsaturated oils, suggesting a role for radical reactions. An excess of carbohydrate decreased the yield, providing a practical means of lowering the formation of HCAs. J. Felton (Lawrence Livermore National Laboratory) had shown earlier that brief microwave heating, which lowers the available creatinine, followed by the usual cooking, sharply decreases the production of HCAs. G. A. Gross (Nestec Ltd. Research Centre, Lausanne, Switzerland), M. G. Knize (Lawrence Livermore National Laboratory), H. Hayatsu (Okayama University, Okayama, Japan), K. Wakabayashi (National Cancer Center Research Institute, Tokyo, Japan), and Felton explored in detail the quantitation and detection of HCAs with a series of rapid *in vitro* tests. This is important, and since most foods contain only small amounts, their accurate measurement is essential. Unlike many other aromatic compounds that are excreted mainly as metabolites, many HCAs are excreted in fair amounts as such, in addition to metabolites. Thus, their intake by humans can be estimated based on the determination of mutagenicity in urine. This procedure is possible because a number of HCAs display enormous specific mutagenicity. The exception is an HCA, abbreviated as PhIP, formed from phenylalanine, that is present in cooked foods in higher amounts than other HCAs but has only low specific mutagenicity. However, other procedures such as the use of monoclonal antibodies and immunoaffinity chromatography, DNA repair tests in hepatocytes, or gas chromatography-mass spectrometry techniques were generally applicable to most HCAs, as reported by S. R. Tannenbaum (M. I. T., Cambridge, MA), by D. S. Davies (Royal Postgraduate Medical School, London, England), and by J. Weisburger (American Health Foundation, Valhalla, NY). Also, Hayatsu has perfected specific techniques to adsorb HCAs from dilute solutions such as urines by means of blue cotton or rayon, with a copper phthalocyanine sulfonate as the active principle. Likewise, porphyrins, hemin, or chlorophyll can reversibly bind HCAs. Hayatsu prepared Sepharose-supported chlorophyllin that could be used for reversible adsorption of HCAs. Urinary mutagenicity from intake of grilled beef was lowered through simultaneous consumption of chlorophyllin, reported by R. H. Dashwood (University of Hawaii, Honolulu, Hawaii), who found that chlorophyllin increased fecal excretion of IQ in rats and lowered the formation of carcinogen-DNA adducts in liver. Chlorophyll forms molecular complexes.

Many reports dealt with the metabolism to reactive products of HCAs. As is true for homocyclic arylamines, a key activation reaction is *N*-oxidation, and the key enzyme is cytochrome P450IA2 in liver, as noted by Felton, R. J. Turesky (Nestec Ltd. Research Centre), F. P. Guengerich (Vanderbilt University School of Medicine, Nashville, TN), J. Alexander (National Institute of Public Health, Oslo, Norway), A. R. Boobis (Royal Postgraduate Medical School), M. E. Mc Manus (Univesity of Queensland, Brisbane, Queensland, Australia), Y. Yamazoe (Keio University School of Medicine, Tokyo, Japan), and F. F. Kadlubar (National Center for Toxicological Research, Jefferson, AR). The *N*-hydroxy compound is conjugated to form a *N*-glucuronide as a transport form and release in target tissues, where further activation by acylation or by sulfation (mostly in liver) can occur.

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[1] This symposium was the Twenty-third International Symposium of the Princess Takamatsu Cancer Research Fund. Organizing Committee (in alphabetical order): R. H. Adamson, J-Å. Gustafsson, N. Ito, M. Nagao, T. Sugimura, K. Wakabayashi, Y. Yamazoe. It was held at the Palace Hotel, Tokyo, Japan, November 10–12, 1992.

[2] The abbreviations used are: HCA, heterocyclic amine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline.

Interestingly,N-OH-PhIP is reactive and can bind to cellular macromolecules. Detoxification through *C*-hydroxylation occurs through cytochrome P450IA1, and there are significant species and organ site differences for this reaction. HCAs can undergo *N*-sulfamate and *N*-glucuronide formation, and since these metabolites are excreted in urine, Tannenbaum and Turesky indicated that their hydrolysis would permit improved detection of intake by humans, through analysis of urines with mutagenicity tests, after splitting of the conjugates. These reactions proceed likewise in monkeys, but E. G. Snyderwine (NCI, Bethesda, MD) observed that IQ and PhIP were converted to diverse metabolites but that MeIQx was excreted in urine unchanged and also that PhIP failed to form *N*-sulfamate and *N*-glucuronide conjugates, in contrast to IQ or MeIQx. In all species, 4′-hydroxylation of PhIP is a detoxification reaction, leading to formation of excretable sulfate and glucuronide conjugates. Interestingly, Wakabayashi found that mutagenic 4′-OH-PhIP was formed during a Jägerstad-type reaction between creatinine, tyrosine, and glucose, and thus, it is found also in cooked foods. Caffeine acetylation may provide an easily measured indicator for extrahepatic and hepatic activation of N-OH-HCA by acetylation. Wakabayashi noted that a new *Salmonella* strain, YG 1024, high in *O*-acetyltransferase, permits more sensitive detection of HCAs and shows that acetylation can be a key activation reaction. Other cytochromes P450, such as IIB1, according to E. Overvik (Karolinska Institute, Huddinge, Sweden) or McManus, in a COS cell expression system, yielded Ames-positive products. The formation of active oxygen by cytochrome *c* reductase was documented by H. Maeda (Kumamoto University Medical School, Kumamoto, Japan) to arise in the presence of HCAs. Powerful inhibition by the drug Furafylline of cytochrome P450IA2 concerned with the metabolic activation of HCAs was observed by Boobis and by Davies who also found that human liver formed mainly the reactive N-OH-HCAs, in contrast to rodents, and especially guinea pigs, who produced chiefly C-OH metabolites (Alexander). According to J-A. Gustafsson (Karolinska Institute, Huddinge, Sweden), indoles from cruciferous vegetables were good inducers of cytochrome P450IA1, perhaps because their dimers fitted well in the dioxin receptor, but the much smaller HCAs were poor inducers of that detoxifying enzyme system.

The reactive N-OH-HCAs are, thus, produced through polymorphic oxidative enzyme systems and further activated through polymorphic *N*-acetyltransferases or sulfotransferases. Humans with high levels may be at high risk of colon cancer (Kadlubar). These key active metabolites can react with cellular macromolecules. The activated metabolites of HCAs react with DNA to form several adducts, visualized most readily by ^{32}P postlabeling of DNA from the liver of rats and monkeys, as reported by a number of speakers. S. Thorgeirsson (NCI, Bethesda, MD) found such adducts with IQ and PhIP but, for unclear reasons, not with MeIQx, but he noted that human liver yields high levels of DNA adducts with MeIQx *in vitro*. One of the main adducts is on C-8 of guanine, and Guengerich suggested that these products might form after first binding to N-7, followed by transfer to the C-8 position, in analogy with observations made with homocyclic arylamines. At the Lawrence Livermore National Laboratory, Turteltaub and colleagues applied accelerator mass spectrometry techniques to quantitate HCA-DNA adducts, with great sensitivity, with doses as low as 1 ng/kg. Application of this instrument promises to yield reliable data with the low exposure levels of HCAs, typical of the human diet.

The genetic consequences of DNA adducts were noted by several speakers, and M. Nagao (National Cancer Center Research Institute, Tokyo, Japan) provided the most comprehensive overview. In the forestomach and ear duct neoplasms induced by MeIQ, there were mutations in the Ha-*ras* gene with a GGC to GTC change at codon 13, and also evidence of *p53* mutations. Ear duct neoplasms in rats giving IQ also had *p53* mutations, and Ha-*ras* mutations. Only 1 of 7 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole-induced colon cancers displayed a GGT to GTT switch at codon 12 of Ki-*ras*, but none in Ha-*ras*, N-*ras*, or *p53*. Likewise, thus far none of the rat colon cancers induced by PhIP or IQ had any obvious mutations in Ha-*ras*, N-*ras*, Ki-*ras*, or *p53*, and the genetic lesion induced by these colon carcinogens remains elusive. Even in PhIP-produced mammary gland cancers, there was no mutation in the transmembrane coding region of the *neu-erb*B-2 oncogene, and in only 3 of 17 was there a GGA to GAA shift at codon 12 of Ha-*ras*, and in 1 of 9, there was an AAG to AAT change in codon 130 of *p53*.

R. H. Adamson (NCI, Bethesda, MD) and associates demonstrated the remarkable carcinogenicity of IQ in nonhuman primates, mostly macaques. With doses of 20 mg/kg 5 times/week, 85% of the monkeys had liver cancer in as little as 2–3 years, with an average latent period of 44 months. No other cancers were observed thus far, and U. P. Thorgeirsson (NCI, Bethesda, MD) found that the liver cancers were mainly well-differentiated trabecular neoplasms, but some had intrahepatic vascular invasion, and some had also metastasis in the lung. In addition, Adamson described lesions in the heart, with myocyte necrosis. Biochemical studies in rats revealed HCA-DNA adducts in the heart (Övervik), and further exploration of this association will be interesting as background for the question whether cooked meat may also affect the cardiovascular system.

N. Ito and T. Shirai (Nagoya City University Medical School, Nagoya, Japan) provided important dose-response relationships. With a 2-year feeding of diets with 400 and 100 ppm of PhIP, colon and mammary tumors were induced, but with 25 ppm no colon cancers and an insignificant yield of mammary gland tumors were found. Also, a rapid, 8-week bioassay involved determination of liver cell foci positive for glutathione *S*-transferase (placental form), after a regimen involving HCA feeding for 6 weeks. Single HCAs at the normal feeding doses were quite active in inducing foci, but importantly, a positive effect was noted with one-tenth of the full dose when a combination of 10 HCAs was tested, suggesting a synergism with a mixture, as might be found in cooked foods. In this context, Weisburger reported that a level of dietary fat, as usually consumed in the Western world, increased the yield of mammary gland cancers in rats fed the low level of 75 ppm IQ. Also, fat stimulated and calcium salt decreased the formation of foci of aberrant crypts in the colon of rats fed PhIP for only 9 weeks. These experiments suggest that noncarcinogenic dietary factors, mimicking the human situation, can modify the effect of HCAs at important target organs. Also, black and green tea, and polyphenols in these teas, sharply lower the mutagenicity of and the induction by IQ and PhIP of DNA repair in liver cells by these carcinogens.

In summary, the Princess Takamatsu Symposium just concluded was one of the most exciting and significant of recent conferences. It was truly multidisciplinary, from epidemiology to molecular biology, with a balanced coverage by an international faculty on an important topic of great contemporary importance, namely the presence of powerful mutagens and carcinogens produced during ordinary cooking, in the food of most people in the world. These affect target organs where cancer in humans is frequent, and thus, the association made in the title "possible human carcinogens" is a conservative, reserved interpretation of the current status. The Symposium has stimulated much discussion, and it is likely that future research will permit using "probable, and even actual" human cancer risks. The International Agency for Research on Cancer would state that for the class of carcinogens discovered by Sugimura and colleagues only 16 years ago, there is sufficient evidence of human cancer causation, as the Agency has already concluded as regards laboratory animals.

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