

# Processing-Induced Mutagens in Muscle Foods

*Thermal processing of muscle foods is important for producing a safe and desirable product, but it can also result in the formation of mutagenic compounds*

Arthur J. Miller

□ MUSCLE FOODS are one of the best sources of nutrients for human growth and maintenance, being especially rich in high-quality protein, vitamins, and minerals. Their contribution to the world population's improved nutritional status experienced during this century, and the concomitant decrease in acute nutritional deficiencies and associated illnesses, reflects glowingly on the biological and logistical availability, the desirability, and the nutrient density of muscle foods. Any alleged negative attributes associated with this food class must be balanced against the contributions that it has made toward our well-being.

Interest in thermally induced mutagens in muscle foods is readily demonstrated by the burgeoning literature that has grown to some 300 technical publications and meeting abstracts since 1977. This demonstrates the current attitude of some researchers, public health officials, and clinicians toward the association between dietary components and chronic diseases, particularly cancer. Citing numerous population studies, several epidemiologists have suggested that indeed diet may be the most important environmental variable in the development of certain cancers (Wynder and Gori, 1977; Doll and Peto, 1981). In addition, the realization that mutations can activate cellular-transforming genes (oncogenes) (Feinberg et al., 1983; Cooper, 1982; Backer et al., 1982) helps support the long debated theory that gene mutation is the first of a series of required events that progress to the development of neoplasia (Weisburger and Williams, 1980; Straus, 1981). Perhaps food-borne mutagens bridge these two diverse observations.

Previously, mutagens have been

observed in muscle foods when subjected to various cooking and processing methods. For example, charcoal-broiled and grilled beef contains benzo(a)pyrene (BaP) (Lijinsky and Shubik, 1964), and nitrosamines can be formed in meat products when nitrite and secondary amines are heated together (Pensabene et al., 1974). It appears now that heating of most, if not all, muscle foods by a variety of cooking methods, can produce mutagens.

Mutagens have been observed in many foods and were classified by Sugimura and Sato (1983) into three general groups, including: naturally occurring, food and feed additives, and processing-induced. This article focuses on the latter group, specifically heat-induced mutagens in muscle foods.

## The Ames Test and the Significance of Mutation

The Ames *Salmonella*/mammalian microsome mutagenicity assay is currently one of the best known and most widely used in vitro test systems to detect mutagenic effects of chemicals. The test detects back mutations at the histidine gene and is about 80–85% accurate in determining carcinogens as mutagens (Brusick, 1983; Maron and Ames, 1983). *Salmonella typhimurium* is used in combination with induced rat liver microsomes (S-9) for the metabolic activation of mutagens, thus incorporating an important aspect of mammalian metabolism. The metabolizing enzymes found in the microsomes are induced in the smooth endoplasmic reticulum when various agents (drugs, envi-

ronmental contaminants) challenge the animal. For the assay, the compound to be tested, the bacterial tester strain, and when required, the metabolizing system are combined directly on to a petri dish containing minimal media. Between  $10^8$ – $10^9$  log-phase bacteria cells are applied per dish and the number of bacteria reverted back to histidine independence is measured by counting the colonies on the plate after incubation at 37° for 48 hr.

The *Salmonella* tester strains are mutants that contain either a base-pair substitution or frameshift mutation (Ames et al., 1975). For the present review only the latter strains will be discussed. Frameshift mutations are introduced in tester strains by the insertion or deletion of bases within the DNA. For example, the site of the histidine mutation in strains TA 1538 and TA 98 is a  $\delta$  (-1) deletion in the normal genome of the gene coding for histidinal dehydrogenase. With this aberration, the strains cannot survive on minimal media without histidine supplementation. Restoration of the deletion by a (+1) insertion (reverse mutation) will restore growth potential.

Mutations arise by specific DNA damaging events, such as a compound's covalent binding to specific bases, intercalations, or both. When the damaged DNA is replicated without being repaired, a mutation is created. This event is significant because it can lead to abnormal changes in protein primary structure by causing incorporation of incorrect amino acids in the peptide sequence. The mutation may be inconsequential or may be significant if biological activity of a critical protein is affected.

The importance of these events to carcinogenesis rests on the

The author is with the Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118

premise that mutations need to occur at critical sites on the genome thereby affecting strategic proteins. Currently, laboratories worldwide are testing the hypothesis that attacks by mutagens on critical DNA targets, and the resulting sequence alterations on oncogenes have the potential to trigger or promote neoplastic transformation. The unfolding mystery of these transforming genes indicates that, in general, their protein products are growth factors required for embryogenesis or repair processes, such as wound-healing. In the differentiated cell, activity is tightly regulated and the genes are normally repressed. Uncontrolled derepression, possibly from mutational events, results in synthesis of the growth factor, followed by rapid cellular growth, and then neoplastic transformation. Perhaps mutagens in foods can trigger this process.

### High Temperature Mutagens

Bacterial mutagens in cooked foods were first observed when Nagao et al. (1977) found that several extracts obtained from broiled fish and beef showed mutagenic activity toward the Ames *Salmonella* strains TA 98 and TA 100 (a base-pair substitution detecting strain) + S-9. Sugimura et al. (1977) found that the mutagenic components were formed from the pyrolysis of D,L-tryptophan and D,L-phenylalanine. Two mutagens identified from the tryptophan pyrolysate were: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1: "P" stands for pyrolysate product) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) (See Fig. 1). Both were demonstrated to be more mutagenic than BaP, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), and N-nitrosodimethylamine (Table 1). The structure of the active component of the D,L-phenylalanine pyrolysate was identified as 2-amino-5-phenylpyridine (Phe-P-1) (Fig. 1). Its specific activity was weaker than the tryptophan pyrolysis products (Table 1). A pyrolysate of glutamic acid contained Glu-P-1 (2-amino-6-methyl-dipyrido[1,2-a:3'-2'-d]imidazole) and Glu-P-2 (2-aminodipyrido[1,2-a:3'-2'-d]imidazole) (Yamamoto et al., 1978). Glu-P-2 was later identified in broiled squid (Yamaguchi et al., 1980) and casein pyrolysate (Yamaguchi et al., 1979). The activity of Glu-P-1 was about 40 times greater than that of Glu-P-2, whose structure differs by only the absence of

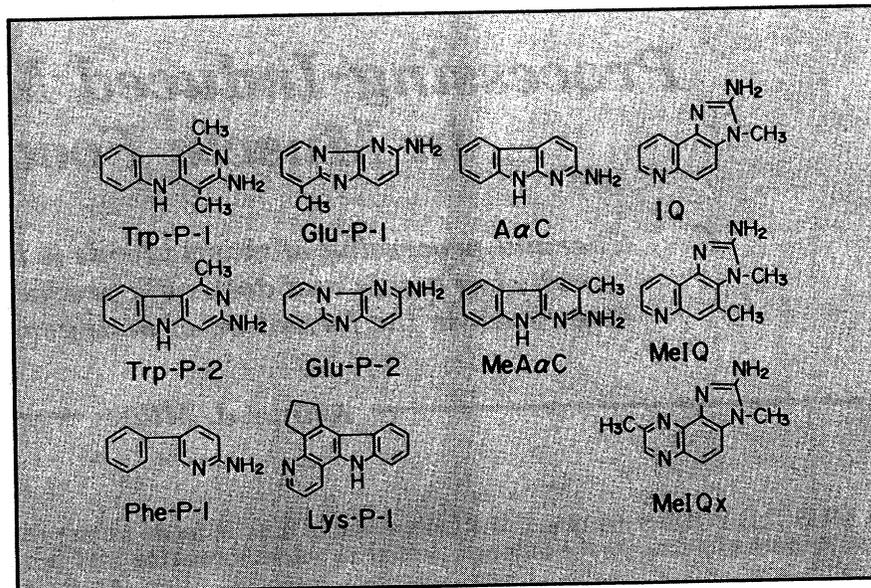


Fig. 1—Heat-Induced Mutagens from muscle foods

one methyl group. Yokota et al. (1981) then found a mutagenic L-ornithine pyrolysis derivative, Orn-P-1 (4-amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene). The mutagenic activity of this compound was found to be about 56,000 TA 98 revertants/ $\mu$ g. Finally, Wakabayashi et al. (1978) observed the formation of Lys-P-1 from a pyrolyzed lysine system. This compound was orders of magnitude lower in mutagenic potency than the Trp or Glu compounds.

Three mutagens were then isolated from protein pyrolysates of soybean globulin, and were determined to be A $\alpha$ C (2-amino- $\alpha$ -carboline), MeA $\alpha$ C (2-amino-3-methyl- $\alpha$ -carboline), and EtA $\alpha$ C (2-amino-3-ethyl- $\alpha$ -carboline) (Matsumoto et al., 1981; Yoshida et al., 1978). Matsumoto et al. (1981) found A $\alpha$ C and MeA $\alpha$ C in grilled ground beef at levels of 650.8 ng/g and 63.5 ng/g, respectively. Grilled chicken and mushrooms contained these compounds, but levels were lower than those of the grilled beef.

Pyrolysis products are likely to be produced by cooking proteinaceous food by direct exposure to the flame (Sugimura et al., 1977), since the temperature required to generate Trp-P-1, -2, and other amino acid pyrolysis mutagens is in excess of 300°C. Therefore, the formation of these compounds requires high cooking temperatures. Pyrolysis produces many reactive fragments by free radical reactions, and these fragments can then condense to form new heterocyclic structures. The formation of Lys-

P-1 from lysine, for example, can be explained by this mechanism. With regard to Trp-P-1 and Trp-P-2, the original indole nucleus of the tryptophan molecule remains intact. Similarly, Phe-P-1 also retains part of the structure of phenylalanine (Matsushima, 1982).

The toxicology of the pyrolysis compounds has been studied extensively, with the results and corresponding references summarized in Table 2. In general, the tryptophan, glutamic acid, and the  $\alpha$ -carboline pyrolysis products demonstrate a consistent positive profile of toxic results. Direct predictors of carcinogenic activity such as  $\gamma$ -glutamyl transpeptidase-positive liver foci and in vitro neoplastic transformation are consistent with the genotoxic data. Studies have also demonstrated multiple species carcinogenicity for Trp-P-1 (3 species), Trp-P-2 (2 species), Glu-P-1, and Glu-P-2 (2 species), and A $\alpha$ C and MeA $\alpha$ C (1 species).

### Moderate Temperature Mutagens

Kasai et al. (1979) detected a mutagen by HPLC in broiled sardines that did not correspond to the then-known compounds: Glu-P-1, Glu-P-2, Lys-P-1, A $\alpha$ C, Trp-P-2, Trp-P-1, or MeA $\alpha$ C. Concomitantly, a number of other laboratories consistently observed that the addition of microsomes to the mutagenicity assay medium produced an increase in the background rate of mutations in frameshift detecting *Salmonella* strains, even in the

absence of mutagenic agents. Commoner et al. (1978a,b) determined that the increased activity resulted from a S-9 dependent component in the nutrient broth used to grow the bacterial cells. This observation led Commoner's group to detect mutagenic activity both in commercial beef extract and then in cooked ground beef (Vithayathil et al., 1978). The observation of mutagens in cooked beef was soon confirmed by others (Spingarn and Weisburger, 1979; Pariza et al., 1979a,b; Felton et al. 1981; Rappaport et al., 1979) who found that moderate temperature cooking, (optimum 190–200°) such as frying and broiling, induced high levels of mutagenic activity.

Temperature is the most important established determinant for mutagen formation in muscle foods. Cooking methods that employ higher heating temperatures induce greater mutagenic activity than low-temperature methods (Miller and Buchanan, 1983b). For example, Nader et al. (1981) showed that broiled beef surfaces contained elevated mutagenic activity when tested with TA 98 + S-9, but microwave-heated beef (up to three times the normal cooking period at 2450 MHz) did not exhibit any genotoxicity. This observation was also reported by Commoner et al. (1978a,b) and Baker et al. (1982) in beef, and Miller and Buchanan (1983b) in pork. However, Taylor et al. (1982) observed little mutagenic activity in ground beef that was deep-fat fried for 3 min. They con-

Table 1—Comparison of Mutagenic Potential of heat-induced mutagens with other known mutagens<sup>a</sup>

Compound	TA 98 revertants / μg
MelQ	661,000
IQ	433,000
MelQ <sub>x</sub>	145,000
Trp-P-2	104,200
Glu-P-1	49,000
Trp-P-1	39,000
Aflatoxin B <sub>1</sub>	6,000
Glu-P-2	1,900
BaP	320
AαC	300
MeAαC	200
Lys-P-1	86
Phe-P-1	41

<sup>a</sup>Adapted from Sugimura (1982)

cluded that, in general, deep-fat fried foods possess low levels of mutagenic activity, and severe frying conditions must be employed to obtain appreciable levels of activity. These results may be due to volatilization (Rappaport et al., 1979) or the inability to extract the mutagens from the oil.

Mutagenicity in fried beef may be affected also by other factors that include fat and water content. In a study by Spingarn et al. (1981) over the range of 5–30% added fat, mutagenicity reached a peak at 10% added fat and subsequently decreased. In a more recent study, however, Bjeldanes et al. (1983)

found that the level of fried beef mutagen was independent of fat content. An explanation for these differences has not been offered. Initial water content of hamburger patties also is important for generation of mutagenic activity. Maximum mutagen formation required an initial water content of 70% by weight and mutagen production was negligible below 30%.

Taylor and coworkers have developed a simple boiling model system derived from lean round steak and have studied the precursors in meat that are involved in mutagen formation. All mutagen precursors in lean round steak are water soluble and free from meat solids (Taylor et al., 1981). Proteolysis of extract residues by papain, trypsin, or chymotrypsin increased mutagen yield 1.7 to 4-fold, indicating that soluble compounds and amino acids released by the enzyme activity were precursors. By another series of experiments (Taylor et al., 1984) these investigators determined that the precursors of the beef mutagen had a molecular weight of <500 daltons and exhibited optimum mutagen formation at pH 4 and 9. The observation of minimum mutagenic activity at pH 7 suggested that manipulation of pH may be a viable means for the control of mutagen formation. The fraction containing the precursors for virtually all of the mutagenic activity represented only 5% of the meat dry weight and 10% of the soluble protein.

Table 2—Qualitative Toxicology Profile of amino acid and protein pyrolysis products (+ = positive; NR = not reported)

Assay	Trp-P-1	Trp-P-2	Glu-P-1	Glu-P-2	Lys-P-1	Orn-P-1	Phe-P-1	AαC	MeAαC
Bacterial point mutation	+ <sup>a,b</sup>	+ <sup>a,b</sup>	+ <sup>b,c</sup>	+ <sup>b,c</sup>	+ <sup>b,d</sup>	+ <sup>e</sup>	+ <sup>a</sup>	+ <sup>b,f</sup>	+ <sup>b,f</sup>
Mammalian in vitro point mutation	+ <sup>b</sup>	+ <sup>g</sup>	NR	NR	+ <sup>h</sup>	NR	NR	NR	NR
Chromosomal aberration	+ <sup>i</sup>	+ <sup>i</sup>	+ <sup>i</sup>	+ <sup>i</sup>	NR	NR	NR	+ <sup>i</sup>	+ <sup>i</sup>
Sister chromatid exchange	+ <sup>i</sup>	+ <sup>i</sup>	+ <sup>i</sup>	NR	NR	NR	NR	+ <sup>i</sup>	NR
Mammalian in vivo specific locus mutation	NR	+ <sup>k</sup>	+ <sup>k</sup>	NR	NR	NR	NR	NR	NR
Mammalian DNA damage in vitro	+ <sup>i</sup>	+ <sup>i</sup>	+ <sup>i</sup>	+ <sup>i</sup>	NR	NR	NR	NR	NR
γ-glutamyl transpeptidase liver foci	+ <sup>m</sup>	+ <sup>m</sup>	+ <sup>m</sup>	+ <sup>m</sup>	+ <sup>n</sup>	NR	NR	+ <sup>n</sup>	+ <sup>n</sup>
Neoplastic transformation in vitro	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>p</sup>	NR	NR	NR	NR	NR	NR
Carcinogenicity (rodent)	+ <sup>q,r,s,v</sup>	+ <sup>q,r,s,u</sup>	+ <sup>b,c</sup>	+ <sup>b,t</sup>	NR	NR	NR	+ <sup>b</sup>	+ <sup>b</sup>

<sup>a</sup>Sugimura et al., 1977

<sup>b</sup>Nagao et al., 1983b

<sup>c</sup>Yamamoto et al., 1978

<sup>d</sup>Wakabayashi et al., 1978

<sup>e</sup>Yokota et al., 1981

<sup>f</sup>Yoshida et al., 1978

<sup>g</sup>Thompson et al., 1982

<sup>h</sup>Takayama and Tanaka, 1983

<sup>i</sup>Ishidate et al., 1981

<sup>j</sup>Tohda et al., 1980

<sup>k</sup>Jensen, 1983

<sup>l</sup>Loury and Byard, 1983

<sup>m</sup>Tamano et al., 1981

<sup>n</sup>Hasegawa et al., 1982

<sup>o</sup>Takayama et al., 1977

<sup>p</sup>Takayama et al., 1979

<sup>q</sup>Yamaizumi et al., 1980

<sup>r</sup>Matsukura et al., 1981a

<sup>s</sup>Ishikawa et al., 1979

<sup>t</sup>Takayama et al., 1984a

<sup>u</sup>Hosaka et al., 1981

<sup>v</sup>Nemoto et al., 1979

**Characterization of Specific Mutagens.** Three potent mutagens in moderately heated foods have been found and well characterized. Two compounds, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), were isolated from broiled fish (See Fig. 1) (Kasai et al., 1980a). In addition, IQ was isolated from beef extract and fried beef. The structure of IQ was elucidated based upon its  $^1\text{H-NMR}$  spectrum, and low- and high-resolution mass spectra (Kasai et al., 1980c). In addition, Yokoyama et al. (1980) determined the crystal and molecular structures of IQ. The molecule exhibited a planar skeletal structure, suggesting that it intercalated between DNA bases, thus promoting the adduct-forming reactions between DNA and the active metabolite of IQ (See Fig. 2). The structure of MeIQ was determined by Kasai et al. (1980b) after comparing the  $^1\text{H-NMR}$  and high resolution mass spectral data for MeIQ with IQ. Later Kasai et al. (1981) also characterized a third potent mutagen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in fried beef (Fig. 1).

Two groups in the United States have confirmed the presence of IQ-type mutagens in heated muscle foods and have further characterized new mutagenic compounds. Hargraves and Pariza (1983) isolated and identified MeIQx, MeIQ, and IQ in Difco beef extract, IQ and MeIQx in food-grade beef extract, and MeIQx and a more polar unidentified mutagen in fried ground beef. Estimates of concentrations of MeIQx were 28 ppb in food-grade beef extract to 0.45 ppb in fried ground beef. Felton et al. (1983) reported that as many as 10 distinct mutagenic compounds were present in ground beef fried at  $250^\circ\text{C}$ . More than 30% of the mutagenic material was identified as MeIQx; IQ contributed less than 12% of the total mutagenicity at  $300^\circ\text{C}$  and less than 4% at lower temperatures. Major unidentified mutagens were observed at molecular ions at  $m/z$  227 ( $\text{C}_{12}\text{H}_{13}\text{N}_5$ ), 209 ( $\text{C}_{13}\text{H}_{11}\text{N}_3$ ), and 176 ( $\text{C}_9\text{H}_{12}\text{N}_4$ ). Two very polar and two nonpolar peaks containing <16% of the total genotoxic activity were not identified. Estimates of mutagen yield suggested that 1 ppb MeIQx, 0.02 ppb IQ, and up to 1.5 ppb additional mutagens were formed from cooked ground beef. In a follow-up study (Knize et al., 1984), the group found MeIQx (1.3

ppb), IQ (0.02 ppb), and unknowns (0.60 ppb) in cooked beef.

Methods of purification of this class of aromatic amines include: solvent extraction, liquid-liquid partitioning, silica gel column chromatography, Sephadex LH-20 column chromatography, TLC, and HPLC. Yamaizumi et al. (1980) developed a GLC method for the quantification of IQ in broiled meat. In that study, only 10% of the total mutagenic activity observed in the product could be attributed to IQ. Hargraves and Pariza (1983) also developed a purification method for mutagens in commercial beef extract using silica gel column chromatography, LH-20 column chromatography, and HPLC (ultra sil-NH<sub>2</sub> and Supelcosil C-18 columns). Detection of the compounds is primarily by ultraviolet absorption (264 nm  $\lambda_{\text{max}}$  for IQ and MeIQ, and 274 nm  $\lambda_{\text{max}}$  for MeIQx).

Extraction and analysis of these trace mutagens is laborious and necessitates that samples undergo multiple chromatographic procedures to near purity, before quantitative analysis is reliable. Confounding factors include: low quantities of the compounds; the presence of nonbiologically active material that coelutes with the IQ compounds upon most chromatographic procedures; lack of a selective detection system; and the tendency for the compounds to adhere to glass thus reducing recoveries. There is a pressing need for a good extraction and isolation method, coupled with a selective quantification procedure, that will permit analysis of these mutagens in complex food substrates.

**Toxicity Studies.** IQ, MeIQ, and MeIQx represent the strongest bacterial mutagens observed to date. The specific mutagenicity of IQ toward TA 98 + S-9 was 433,000 revertants/ $\mu\text{g}$ . IQ was much more potent than Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, and AFB<sub>1</sub> (Table 1). MeIQ and MeIQx were shown to be very potent bacterial mutagens with activity toward TA 98 + S-9 of 661,000 revertants/ $\mu\text{g}$  and 145,000 revertants/ $\mu\text{g}$ , respectively (Sugimura, 1982). However, a number of studies indicate that the IQ compounds, while still biologically active, are not as potent in other genetic assays as in *Salmonella*. IQ was determined to be mutagenic in mammalian cells in vitro, but less so than Trp-P-2 (Thompson et al., 1983; Nakayasu et al., 1983). Yet, Takayama and Tanaka (1983)

found that IQ and MeIQ were not genotoxic in their in vitro mammalian mutagenicity assay. The authors suggested that the discrepancy may be due to differences in the genetic markers used for selection of mutation. Recently, Bird and Bruce (1984) determined that IQ and MeIQ caused high levels of nuclear aberrations in colonic crypt cells when fed to rats. Furthermore, Cortesi and Dolara (1983) showed that IQ induced neoplastic transformation of Balb3T3 mouse embryo fibroblasts at concentrations of 1, 5, and 15 ng/ml.

Carcinogen determination experiments have shown that IQ is moderately carcinogenic in mice, producing tumors in the liver, forestomach, and lungs when fed at 300 ppm (Ohgaki et al., 1984). In a similar rat study, tumors were observed in the zymbal gland of the ear duct and the intestines. Mammary tumors also were observed (Takayama et al., 1984b). Sugimura (1983) suggested that the discrepancy between the high mutagenic and moderate carcinogenic potency of IQ may be idiosyncratic to the *Salmonella*, which is high in guanines and cytosines at the mutated sites. IQ has a high affinity for guanine, and runs of this "hot spot" would make the strain particularly sensitive to this compound. Results of this nature emphasize the fact that the Ames test provides more reliable qualitative than quantitative predictability for carcinogens. Carcinogenicity studies on MeIQ and MeIQx are currently under investigation in Japan.

**Model Systems.** In an attempt to explain how these thermally induced mutagens form, many investigators have utilized model systems. For example, a number of investigators have demonstrated that mutagens can be generated in model Maillard reactions. Spingarn and Garvie (1979) demonstrated that refluxed reducing sugars (especially rhamnose, xylose, glucose, and galactose) plus an ammonium salt resulted in the formation of strong mutagenic activity. These reactions were base-catalyzed and were inhibited by the antioxidant propyl gallate. Shibamoto et al. (1981) showed that maltol and ammonia (heated at  $100^\circ\text{C}$  for 5 hr) produced mutagens detectable by TA 98 + S-9. The investigators determined that alkyl-pyridine derivatives induced the mutagenic activity.

Mutagenicity was observed in heated mixtures of creatine and

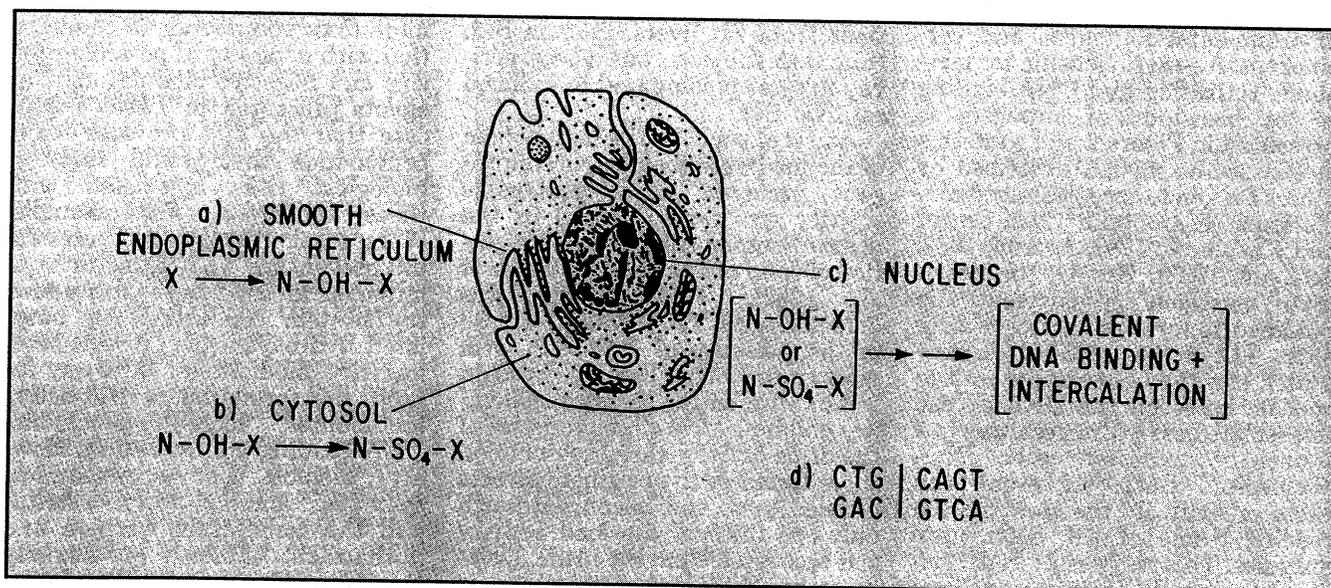


Fig. 2—Metabolic Activation of the heat-induced mutagens occurs primarily in the (a) smooth endoplasmic reticulum, where CP-448 enzymatically forms the hydroxyl derivative; (b) further metabolism can occur in the cytosol to form the sulfate ester; (c) in the nucleus, the electrophile covalently binds to the guanine residues on DNA and can (d) intercalate adjacent base pairs (solid line), thus resulting in a shift in the polymerase reading frame and causing a mutation to occur upon DNA replication.

glucose (above 150°C for 1–2 hr), as detected by TA 98 + S-9, while no activity was observed without glucose present in the reaction mixture (Yoshida and Okamoto, 1980a). Since creatine and glucose are common components of meat, they may provide a significant contribution to the formation of mutagens during the heating of meat (Yoshida and Okamoto, 1980b).

Since cooking procedures range widely from mild heating to strong heating, the formation of mutagens during cooking may be due to both browning and pyrolysis. Yoshida and Okamoto (1982) found that mutagenic activity could be detected in the pyrolysis product of the organic ammonium salts of malate, citrate, tartarate, and oxalate (550°C for 1 min) using TA 98 + S-9 as the indicator organism. Ohe (1982) tested 21 nitrogen-containing compounds that had been pyrolyzed at 300–600°C for 3 min for mutagenic activity using TA 100 and TA 98, in the presence of S-9. Methylguanidine, agmatine, dihydrouracil, dimethylamine, diethylamine, trimethylamine, triethylamine, pyrrolidine, morpholine, sarcosine, piperazine, piperidine, spermine, and spermidine, showed mutagenic activity, especially with TA 98.

Recently, studies have focused on model systems that included mixtures of nitrogenous components, such as amino acids and creatine derivatives, with sugars. Jägerstad

et al. (1982, 1984) studied the formation of IQ compounds from a creatinine, glucose, and amino acid reflux system. Similarly, IQ was generated from a heated mixture of proline and creatine (Yoshida et al., 1984). Yoshida and Fukuhara (1982) had previously observed mutagenic responses from mixtures of heated creatine and cystine, threonine, phenylalanine, methionine, tryptophan, valine, proline, or serine (200°C) using TA 98 + S-9. Free cystine, lysine, and tryptophan are not normally contained in meat; therefore, the authors concluded that the formation of mutagens by the reaction between creatine and other amino acids which are present would be expected during the cooking of beef.

**Mutagens in Other Cooked Foods.** Krone and Iwaoka (1981) suggested that mutagens are probably formed not only from beef, but also during the heating of other foods. Pariza et al. (1979a) found that canned chicken broth and beef broth exhibited moderately high levels of TA 98 mutagenic activity; crackers, corn flakes, rice cereal, and bread crust had low levels of mutagenic activity; bread crumbs, toast (surface), and coconut cookies had low-marginal levels of activity. Krone and Iwaoka (1981) found mutagenic activity toward TA 1537, TA 1538, and TA 98 in fish fried at 190°C. Levin et al. (1981) used a modification of the *Salmonella* assay with TA 98 and found that

many commercial preparations had significant levels of mutagenic activity. These included dehydrated products such as: beef broth, vegetable beef soup mix, seasoning, beef bouillon cubes, beef barley soup, and oxtail soup. Other products showing activity were canned chicken broth and evaporated milk.

A number of reports have appeared in the literature concerning formation of mutagens in cooked pork and pork products. Gocke et al. (1982) found mutagenic activity in pan-fried sausages in seven test systems. Bjeldanes et al. (1982) found that many protein-rich foods normally consumed by Americans, including pork products, formed genotoxic components when cooked, as detected by TA 1538 + S-9. Lin et al. (1982) found that boiled pork showed Ames test positive results with TA 1538 and TA 98 + S-9. Our findings on the detection of mutagens in fried bacon, both nitrite-free and -treated, have been published recently (Miller and Buchanan, 1983a,b). Övervik et al. (1984) have observed similar mutagenic activity in pan-broiled pork.

### Metabolism

The metabolism of the IQ compounds present many similarities with the pyrolysis products and therefore will be discussed collectively. Biological activation and DNA binding is a hallmark of many

environmental mutagens and carcinogens. A generic scheme for metabolic activation and DNA binding of the heat-induced mutagens is presented in Figure 2 and conforms closely to the models described by others for genotoxic agents and carcinogens (Hemminki, 1983; Singer and Kusnierek, 1982; Autrup, 1982; Jefcoate, 1983). Briefly, the proximate mutagen is enzymatically modified to form the active metabolite. The active species (electrophile), can then covalently bind to specific bases on the DNA or can intercalate base-pairs, resulting in a frameshift mutation upon DNA replication. Such an event can cause a shift in the downstream reading frame of the polymerase enzymes. This may result in permanently altered protein functions induced by changes in the nucleotide and, thus, amino acid succession.

Considerable knowledge has been gained in the understanding of biological activation of the amino acid and protein pyrolysis products. Early work indicated a strict requirement for metabolism by S-9. Trp-P-2 is metabolized to its active form by a cytochrome (C)P-448 monooxygenase system in the microsomal S-9 fraction (Kato et al., 1983; Kawajiri et al., 1983; Kamataki et al., 1983). Strongly basic amines are preferred substrates for CP-448, whereas the less basic amines are preferred substrates for the CP-450 system (Kato et al., 1983). Then, reports from the United States and Japan demonstrated the binding of Trp-P-2 to DNA with activation by isolated liver microsomes (Hashimoto et al., 1978; Nemoto et al., 1979; Nebert et al., 1979) and cytosolic enzymes. Guanine residues were the binding sites on DNA (Hashimoto et al., 1979). Presently, active metabolites of Trp-P-2, Glu-P-1, and A $\alpha$ C were shown to be the N-hydroxy forms (Yamazoe et al., 1980a,b; Hashimoto et al., 1980a,b; Mita et al., 1981a,b; Niwa et al., 1982) (see Fig. 2). Nagao et al. (1983a) demonstrated that sulfate esters were the ultimate forms for Glu compounds, while the Trp and  $\alpha$ -carbolines were most active as the hydroxy derivative.

Prior to the isolation of the IQ compounds from fried meat, studies on the metabolism of the cooked meat mutagens were confined to extracts of food. Dolara et al. (1980) found that the in vitro activation of mutagens isolated from beef extract required S-9 frac-

tions from rat livers. Human S-9, from four subjects, was a poor activation system compared to induced rat liver S-9.

A new element in the metabolism of the moderate temperature mutagens occurred when mutagenic activity was demonstrated in the urine of human subjects after ingestion of fried pork or bacon (Baker et al., 1982). This activity was detected with TA 1538 and TA 98 in the presence of liver S-9, with most mutagenicity recovered within 2-4 hr after eating the meal. Chemical and biological characteristics of the urine activity closely resembled those found in the original extracts of fried pork and bacon, although only one-third of the food activity was recovered from the urine. Dolara et al. (1984) qualitatively confirmed the increase in urinary mutagenic activity after a fried meat meal. The overall excretion of mutagens in the urine, however, was much lower than in the Baker et al. (1982) study. The authors suggested that if meat mutagens have a genetic effect in humans it should not occur in organs and tissues distal to the digestive tract.

Once the IQ compounds were characterized, and small quantities of the difficult-to-synthesize compounds became available, various investigators began to report on their distribution, metabolism, and excretion. Sjödin and Jägerstad (1984) reported on the absorption and excretion of <sup>14</sup>C-labeled IQ and MeIQ in rats. Greater than 90% of a gavage dose was removed from the body within 24 hr, via the urine and feces. Gayda and Pariza (1983) studied the activation characteristics of IQ and found that intact cultured hepatocytes produced 10 times the amount of bacterial mutagenicity as disrupted or freshly isolated hepatocytes. The proximate active metabolite of IQ was shown to be the hydroxylamine (Okamoto et al., 1981) and was activated by cytochrome P-448 similar to the amino acid pyrolysates (Watanabe et al., 1982). The ultimate mutagenic form of the IQ compounds is the sulfate ester (Nagao et al., 1983a; Loretz and Pariza, 1984).

### Modification of Mutagenic Activity

Man is seldom exposed to a single mutagen or carcinogen, but rather to complex mixtures of several chemical and/or physical agents. Mixtures may consist of one or

more toxic compounds in combination with a variety of possible positive and negative modulators. The agents presented in Table 3 indicate that the diet provides many compounds that would suppress biological risks associated with these mutagens. For example, Wang et al. (1982) were successful in preventing the formation of mutagen in fried beef with added soy protein concentrate or butylated hydroxyanisole (BHA). Most negative modulators, however, inhibit the formation of the active metabolites, thereby precluding DNA binding and mutation. However,  $\beta$ -thiols, such as cysteamine, cysteine, and N-acetyl-cysteine, and the comutagens harmon and norharmon, enhance genotoxicity (Negishi and Hayatsu, 1979; Deflora et al., 1984; Sugimura et al., 1977). Hayatsu et al. (1981) found that oleic acid inhibited mutagenicity in the cooked beef basic fraction. Interestingly, Pariza et al. (1979b) presented data to suggest that a mutagenic inhibitory factor was present in uncooked hamburger and ground pork. Tsuda et al. (1983) observed that IQ was effectively degraded by tap water or 1.5 ppm hypochlorite with a loss of mutagenic activity. Antimutagens were reviewed recently by Ames (1983).

### Use Heat Wisely to Reduce Mutagen Formation

The overall effect of consuming the processing-induced mutagens depends on a balance of the large number of interactions present among the components, the metabolism and distribution of the mutagens, and the target cell. Therefore, while studies on individual components of complex mixtures such as foods are important, their in vivo effects are dependent upon a plethora of interacting variables. As such, Sugimura (1978) has indicated that by the age of 50, everyone has eaten roughly 10 tons dry weight of food. Although the total concentrations of mutagens in foods are probably very low, their accumulated intake could have the potential to cause genetic damage of cells. It is suspected, however, that the actual amounts of mutagens consumed daily are small enough to be inactivated. For example, the observation that IQ is inactivated by hypochlorite implies that most drinking water consumed during a meal may effectively prevent

any adverse effects from this mutagen.

For the present, the judicious application of heat to a given food product is the most effective and practical means to minimize mutagen formation. Fat and water levels, pH, or dietary manipulation may prove to be effective, but more research is required to substantiate the limited observations. Processors must recognize the potential for generation of genotoxic agents from endogenous precursors in heated muscle foods. They should determine and adopt the lowest level of thermal processing necessary to achieve a safe and desirable product. This may be achieved by use of minimal amounts of heat for established processes or by substitution of heating methods. For example, microwave heat and steam are advantageous over direct flame heating or cooking on hot metal surfaces. Similarly, consumers should consider modifying their cooking practices to use lower temperatures when cooking and to prevent overcooking food surfaces. Establishment of these guidelines can suppress mutagen formation significantly.

Ultimately, the risk of exposure to the cooking-induced mutagens must be weighed against the benefit of the consumption of the foods in which they are contained. To provide this information, a routine selective and quantitative method of analysis needs to be developed to permit an exposure estimate of such compounds. In addition, toxicological evaluations should continue, thus allowing an accurate assessment of toxic potency. These two factors, exposure and potency, can be used then to determine realistic risk. However, this must be balanced against the desirable attributes of muscle food consumption.

Food is more than metabolic fuel. It has physiological, psychological, social, cultural, and aesthetic associations that merge to form a gestalt that people engender and maintain. The contribution of any food toward an individual's well-being is as complex as that individual. Within this context, the benefits of consuming foods containing mutagens may outweigh the risks. The effects of exposure to food-borne mutagens, such as those observed in heated muscle foods, should be studied further, not only to assess potential health risks, but to also devise means for their further reduction or total elimination.

Table 3—Modulation of Activity of heat-induced mutagens

Modulator	Effect on mutagenic activity <sup>a</sup>	Reference
Human saliva	—	Nishioka et al., 1981
Nitrite	— <sup>b</sup>	Yoshida and Matsumoto, 1978; Tsuda et al., 1980, 1981
Cobaltous chloride	—	Mochizuki and Kata, 1982
Germanium oxide	—	Kada et al., 1984
Tannic acid	—	Kada et al., 1984
Polyphenols	—	Fukuhara et al., 1981
Porphyrin-containing compounds	—	Arimoto et al., 1980
Unsaturated fatty acids	—	Saito et al., 1983
Peroxidases	—	Yamada et al., 1979
Retinol (vitamin A)	—	Busk et al., 1982
$\beta$ -thiols	—	Negishi and Hayatsu, 1979; DeFlora et al., 1984
Xanthine derivatives	—	Yamaguchi and Nakagawa, 1983
Soy protein concentrate	—	Wang et al., 1982
BHA	—	Wang et al., 1982
Hypochlorite	—	Tsuda et al., 1983
Beef components	—	Pariza et al., 1979b
Vegetable extracts	—	Morita et al., 1978; Inoue et al., 1981
Harmon and norharmon	+	Sugimura et al., 1977
Organic solvents	+	Arimoto et al., 1982

<sup>a</sup>— = inhibits mutagenic activity.

+ = increases mutagenic activity.

<sup>b</sup>Amino acid pyrolysis products; IQ compounds are resistant to nitrite treatment.

## References

- Ames, B.N., McCann, J., and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31: 347.
- Ames, B.N. 1983. Dietary carcinogens and anticarcinogens. *Science* 221: 1256.
- Arimoto, S., Ohara, Y., Namba, T., Negishi, T., and Hayatsu, H. 1980. Inhibition of the mutagenicity of amino acid pyrolysis products by hemin and other biological pyrrole pigments. *Biochem. Biophys. Res. Comm.* 92: 662.
- Arimoto, S., Nakano, N., Ohara, Y., Tanaka, K., and Hayatsu, H. 1982. A solvent effect on the mutagenicity of tryptophan-pyrolysate mutagens in the *Salmonella*/mammalian microsome assay. *Mutat. Res.* 102: 105.
- Astrup, H. 1982. Carcinogen metabolism in human tissues and cells. *Drug Metabolism Rev.* 13: 603.
- Backer, J.M., Boerzig, M., and Weinstein, I.B. 1982. When do carcinogen-treated 10T 1/2 cells acquire the commitment to form transformed foci? *Nature* 299: 458.
- Baker, R., Arlauskas, A., Bonin, A., and Angus, D. 1982. Detection of mutagenic activity in human urine following fried pork or bacon meals. *Canc. Lett.* 16: 81.
- Bird, R.P., and Bruce, W.R. 1984. Damaging effect of dietary components to colonic epithelial cells in vivo: effect of mutagenic heterocyclic amines. *J. Nat. Canc. Instit.* 73: 237.
- Bjeldanes, L.F., Morris, M.M., Felton, J.S., Healy S., Stuermer, D., Berry, P., Timourian, H., and Hatch, F.T. 1982. Mutagens from the cooking of food. II. Survey by Ames/*Salmonella* test of mutagen formation in the major protein-rich foods of the American diet. *Fd. Chem. Toxic.* 20: 357.
- Bjeldanes, L.F., Morris, M.M., Timourian, H., and Hatch, F.T. 1983. Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. *J. Agric. Food Chem.* 31: 18.
- Brusick, D. 1983. Mutagenicity and carcinogenicity correlations between bacteria and rodents. In "Cellular Systems for Toxicity Testing," ed. G.M. William, V.C. Dunkel, and V.A. Ray. *Ann. NY Acad. Sci.* 407: 164.
- Busk, L., Ahlberg, U.G., and Albanus, L. 1982. Inhibition of protein pyrolysate mutagenicity by retinol (vitamin A). *Fd. Chem. Toxic.* 20: 535.
- Commoner, B., Vithayathil, A.J., and Dolara, P. 1978a. Mutagenic analysis as a means of detecting carcinogens in foods. *J. Food Protect.* 41: 996.
- Commoner, B., Vithayathil, A.J., Dolara, P., Nair, S., Madyastha, P., and Cuca, G.C. 1978b. Formation of mutagens in beef and beef extract during cooking. *Science* 201: 913.
- Cooper, G.M. 1982. Cellular transforming genes. *Science* 218: 801.
- Cortesi, E., and Dolara, P. 1983. Neoplastic transformation of Balb 3T3 mouse embryo fibroblasts by the beef extract mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. *Canc. Lett.* 20: 43.
- DeFlora, S., Benniselli, C., Zanacchi, P., Camoirano, A., Morelli, A., and DeFlora, A. 1984. In vitro effects of N-acetyl cysteine on the mutagenicity of direct acting compounds and procarcinogens. *Carcinogenesis* 5: 505.
- Dolara, P., Barale, R., Mazzoli, S., and Benetti, D. 1980. Activation of the mutagens of beef extract in vitro and in vivo. *Mutat. Res.* 79: 213.
- Dolara, P., Caderni, G., Salvadori, M., Trin-

- gale, L., and Lodovici, M. 1984. Urinary mutagens in humans after fried pork and bacon meals. *Canc. Lett.* 22: 275.
- Doll, R., and Peto, R. 1981. The causes of cancer: quantitative estimate of avoidable risks of cancer in the United States today. *J. Nat. Canc. Instit.* 66: 1191.
- Feinberg, A.P., Vogelstein, B., Droller, M.J., Baylin, S.B., and Nelkin, B.D. 1983. Mutation affecting the 12th amino acid of the C-Ha-ras oncogene product occurs infrequently in human cancer. *Science* 220: 1175.
- Felton, J.S., Healy, S., Stuermer, D., Berry, C., Timourian, H., Hatch, F.T., Morris, M., and Bjeldanes, L.F. 1981. Mutagens from the cooking of food. I. Improved extraction and characterization of mutagenic fractions from cooked-ground beef. *Mutat. Res.* 88: 33.
- Felton, J.S., Knize, M.G., Wood, C., Wuebbles, B.J., Healy, S.K., Stuermer, D.H., Bjeldanes, L.F., Kimble, B.J., and Hatch, F.T. 1983. Isolation and characterization of new mutagens from fried ground beef. *Carcinogenesis* 5: 95.
- Fukuhara, Y., Yoshida, D., and Goto, F. 1981. Reduction of mutagenic products in the presence of polyphenols during pyrolysis of protein. *Agric. Biol. Chem.* 45: 1061.
- Gayada, D.P. and Pariza, M.W. 1983. Activation of 2-amino-3-methylimidazo[4,5-f]quinoline and 2-aminofluorene for bacterial mutagenesis by primary monolayer cultures of adult rat hepatocytes. *Mutat. Res.* 118: 7.
- Gocke, E., Eckhardt, K., King, M.-T., and Wild, D. 1982. Mutagenicity study of fried sausages in *Salmonella*, *Drosophila*, and mammalian cells in vitro and in vivo. *Mutat. Res.* 101: 293.
- Hargraves W.A. and Pariza, M.W. 1983. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. *Canc. Res.* 43: 1467.
- Hasegawa, R., Tsuda, H., Ogiso, T., Ohshima, M., and Ito, N. 1982. Initiating activities of pyrolysis products of L-lysine and soybean globulin assessed in terms of the induction of  $\gamma$ -glutamyl transpeptidase-positive foci in rat liver. *Gann* 73: 158.
- Hashimoto, Y., Takeda, K., Shudo, K., Okamoto, T., Sugimura, T., and Kosuge, T. 1978. Rat liver microsome-mediated binding to DNA of 3-amino-1-methyl-5H-pyrido[4,3-b]indole, a potent mutagen isolated from tryptophan pyrolysate. *Chem.-Biol. Interactions* 23: 137.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1979. Structural identification of a modified base in DNA covalently bound with mutagenic 3-amino-1-methyl-5H-pyrido[4,3-b]indole. *Chem. Pharm. Bull.* 27: 1058.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1980a. Activation of a mutagen, 3-amino-1-methyl-5H-pyrido[4,3-b]indole. Identification of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole and its reaction with DNA. *Biochem. Biophys. Res. Comm.* 96: 355.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1980b. Metabolic activation of a mutagen, 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole. Identification of 2-hydroxyamino-6-methyldipyrido[1,2-a:3',2'-d]-imidazole and its reaction with DNA. *Biochem. Biophys. Res. Comm.* 92: 971.
- Hayatsu, H., Arimoto, S., Togawa, K., and Makita, M. 1981. Inhibitory effect of the ether extract of human feces on activities of mutagens: Inhibition by oleic and linoleic acids. *Mutat. Res.* 81: 287.
- Hemminki, K. 1983. Nucleic acid adducts of chemical carcinogens and mutagens *Arch. Toxicol.* 52: 249.
- Hosaka, S., Matsushima, T., Hirono, I., and Sugimura, T. 1981. Carcinogenic activity of 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), a pyrolysis product of tryptophan. *Canc. Lett.* 13: 23.
- Inoue, T., Morita, K., and Kada, T. 1981. Purification and properties of a plant desmutagenic factor for the mutagenic principle of tryptophan pyrolysate. *Agric. Biol. Chem.* 45: 345.
- Ishidate, M. Jr., Sofuni, T., and Yoshikawa, K. 1981. Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and carcinogens. *Gann Monogr. Canc. Res.* 27: 95.
- Ishikawa, T., Takayama, S., Kitagawa, T., Kawachi, T., Kinebuchi, M., Matsukura, N., Uchida, E., and Sugimura, T. 1979a. In vivo experiments on tryptophan pyrolysis products. In "Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis." ed. E. C. Miller, p. 159. Japan Sci. Soc. Press, Tokyo/Univ. Park Press, Baltimore.
- Jägerstad, M., Laser, A., Reuterswärd, R., Öste, R., Dahlqvist, A., Grivas, S., Olsson, K., and Nyhammar, T. 1982. Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In "Maillard Reactions in Foods and Nutrition." ed. G.R. Walls and M.S. Feather. ACS Symposium Series 215: 507.
- Jägerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., and Sugimura, T. 1984. Formation of 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline in a model system by heating creatinine, glycine, and glucose. *Mutat. Res.* 26: 239.
- Jefcoate, C.R. 1983. Integration of xenobiotic metabolism in carcinogen activation and detoxification. In "Biological Basis of Detoxication," ed. J. Caldwell and W.B. Jakob, p. 31. Academic Press, New York.
- Jensen, N.J. 1983. Pyrolytic products from tryptophan and glutamic acid are positive in the mammalian spot test. *Canc. Lett.* 20: 241.
- Kada, T., Mochizuki, H., and Miyao, K. 1984. Antimutagenic effects of germanium oxide on Trp-P-2 induced frameshift mutation in *Salmonella typhimurium* TA98 and TA1538. *Mutat. Res.* 125: 145.
- Kamataki, T., Maeda, K., Yamazoe, Y., Matsuda, N., Ishii, K., and Kato, R. 1983. A high-spin form of cytochrome P-450 highly purified from polychlorinated biphenyl-treated rats. Catalytic characterization and immunochemical quantitation in liver microsomes. *Mol. Pharmacol.* 24: 146.
- Kasai, H., Nishimura, S., Nagao, M., Takahashi, Y., and Sugimura, T. 1979. Fractionation of a mutagenic principle from broiled fish by high-pressure liquid chromatography. *Canc. Lett.* 7: 343.
- Kasai, H., Nishimura, S., Wakabayashi, K., Nagao, M., and Sugimura, T. 1980a. Chemical synthesis of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), a potent mutagen isolated from broiled fish. *Proc. Japan Acad.* 56: 382.
- Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., and Nishimura, S. 1980b. Structure and chemical synthesis of ME-IQ, a potent mutagen isolated from broiled fish. *Chem. Lett.* 1391.
- Kasai, H., Yamaizumi, Z., and Nishimura, S. 1980c. A potent mutagen in broiled fish. Part 1. 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline. *J.C.S. Perkin I.* 2290.
- Kasai, H., Shiomi, T., Sugimura, T., and Nishimura, S. 1981. Synthesis of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (Me-IQx), a potent mutagen isolated from fried beef. *Chem. Lett.* 675.
- Kato, R., Kamataki, T., and Yamazoe, Y. 1983. N-hydroxylation of carcinogenic amines. *Environ. Health Persp.* 49: 21.
- Kawajiri, K., Yonekawa, H., Gotoh, O., Watanabe, J., Igarashi, S., and Tagashira, Y. 1983. Contributions of two inducible forms of cytochrome P-450 in rat liver microsomes to the metabolic activation of various chemical carcinogens. *Canc. Res.* 43: 819.
- Knize, M.G., Wuebbles, B.J., Healy, S.K., Wood, C.W., Felton, J.S., and Hatch, F.T. 1984. Separation and purification of mutagens from cooked beef using XAD-2 resin adsorption followed by preparative, analytical and semi-micro HPLC, Paper No. 880. Proceedings of the Pittsburgh Conf., Atlantic City, N.J.
- Krone, C.A., and Iwaoka, W.T. 1981. Mutagen formation during the cooking of fish. *Canc. Lett.* 14: 93.
- Levin, D.E., Blunt, E., and Levin, R.E. 1981. Direct detection of mutagenic activity in food products with a modified fluctuation test. *Mutat. Res.* 85: 309.
- Lijinsky, W., and Shubik, P. 1964. Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science* 145: 53.
- Lin, J.-Y., Lee, H., and Huang, H.-I. 1982. Formation of mutagens in boiled pork extract. *Food Chem. Toxic.* 20: 531.
- Loretz, L. and Pariza, M.W. 1984. Effect of glutathione levels, sulfate levels, and metabolic inhibitors on covalent binding of 2-amino-3-methylimidazo[4,5-f]quinoline and 2-acetylaminofluorene to cell macromolecules in primary monolayer cultures of adult rat hepatocytes. *Carcinogenesis* 5: 895.
- Lourey, D.J., and Byard, J.L. 1983. Aroclor 1254 pretreatment enhances the DNA repair response to amino acid pyrolysate mutagens in primary cultures of rat hepatocytes. *Canc. Lett.* 20: 283.
- Maron, D.M. and Ames, B.N. 1983. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* 113: 173.
- Matsukura, N., Kawachi, T., Morino, K., Ohgaki, H., and Sugimura, T. 1981. Carcinogenicity in mice of mutagenic compounds from a tryptophan pyrolysate. *Science* 213: 346.
- Matsumoto, T., Yoshida, D., and Tomita, H. 1981. Determination of mutagens, amino- $\alpha$ -carbolines in grilled foods and cigarette smoke condensate. *Canc. Lett.* 12: 105.
- Matsushima, T. 1982. Mechanisms of conversion of food components to mutagens and carcinogens. In "Molecular Interrelations of Nutrition and Cancer." ed. M.S. Arnott, J. Van Eys, and Y.M. Wang, p. 35. Raven Press, New York.
- Miller, A.J. and Buchanan, R.L. 1983a. Detection of genotoxicity in fried bacon by the *Salmonella*/mammalian microsome mutagenicity. *Food Chem. Toxic.* 21: 319.
- Miller, A.J. and Buchanan, R.L. 1983b. Reduction of mutagen formation in cooked nitrite-free bacon by selected cooking treatments. *J. Food Sci.* 48: 1772.
- Mita, S., Ishii, K., Yamazoe, Y., Kamataki, T., Kato, R., and Sugimura, T. 1981a. Evidence for the involvement of N-hydroxylation of 3-amino-1-methyl-5H-pyrido[4,3-b]indole by cytochrome P-450 in the covalent binding to DNA. *Canc. Res.* 41: 3610.
- Mita, S., Yamazoe, Y., Kamataki, T., and Kato, R. 1981b. Metabolic activation of a tryptophan pyrolysis product, 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) by isolated rat liver nuclei. *Canc. Lett.* 14: 261.
- Mochizuki, H. and Kada, T. 1982. Antimu-

- tagenic action of cobaltous chloride on Trp-P-1-induced mutations in *Salmonella typhimurium* TA 98 and TA 1538. *Mutat. Res.* 95: 145.
- Morita, K., Hara, M., and Kada, T. 1978. Studies on natural desmutagens; screening for vegetable and fruit factors active in inactivation of mutagenic pyrolysis products from amino acids. *Agric. Biol. Chem.* 42: 1235.
- Nader, C.J., Spencer, L.K., and Weller, R.A. 1981. Mutagen production during pan-broiling compared with microwave irradiation of beef. *Canc. Lett.* 13: 147.
- Nagao, M., Honda, M., Seino, Y., Yahagi, T., and Sugimura, T. 1977. Mutagenicities of smoke condensates and the charred surface of fish and meat. *Canc. Lett.* 2: 221.
- Nago, M., Fujita, Y., Wakabayashi, K., and Sugimura, T. 1983a. Ultimate forms of mutagenic and carcinogenic heterocyclic amines produced by pyrolysis. *Biochem. Biophys. Res. Comm.* 114: 626.
- Nagao, M., Sato, S., and Sugimura, T. 1983b. Mutagens produced by heating foods. In "The Maillard Reaction in Foods and Nutrition," ed. G. R. Waller and M. S. Feather ACS Symposium Series 215: 521.
- Nakayasu, M., Nakasato, F., Sakamoto, H., Terada, M., and Sugimura, T. 1983. Mutagenic activity of heterocyclic amines in Chinese hamster lung cells with diphtheria toxin resistance as a marker. *Mutat. Res.* 118: 91.
- Nebert, D.W., Bigelow, S.W., Okey, A.B., Yahage, T., More, Y., Nagao, M., and Sugimura, T. 1979. Pyrolysis products from amino acids and protein: highest mutagenicity requires cytochrome P<sub>1</sub>-450. *Proc. Nat. Acad. Sci.* 76: 5929.
- Negishi, T., and Hayatsu, H. 1979. The enhancing effects of cysteine and its derivatives on the mutagenic activities of the tryptophan-pyrolysis products Trp-P-1 and Trp-P-2. *Biochem. Biophys. Res. Comm.* 88: 97.
- Nemoto, N., Kisumi, S., Takayama, S., Nagao, M., and Sugimura, T. 1979. Metabolic activation of 3-amino-5H-pyrido[4,3-b]indole, a highly mutagenic principle in tryptophan pyrolysate, by rat liver enzymes. *Chem.-Biol. Interactions* 27: 191.
- Nishioka, H., Nishi, K., and Kyokane, K. 1981. Human saliva inactivates mutagenicity of carcinogens. *Mutat. Res.* 85: 323.
- Niwa, T., Yamazoe, Y., and Kato, R. 1982. Metabolic activation of 2-amino-9H-pyrido[2,3-b]indole by rat-liver microsomes. *Mutat. Res.* 95: 159.
- Ohe, T. 1982. Mutagenicity of pyrolysates from guanidine, ureide, secondary amines and polyamines found by the *Salmonella*/mammalian-microsome test. *Mutat. Res.* 101: 175.
- Ohgaki, H., Kusama, K., Matsukura, N., Marino, K., Hasegawa, H., Sato, S., Takayama, S., and Sugimura, T. 1984. Carcinogenicity in mice of a mutagenic compound 2-amino-3-methylimidazo[4,5-f]quinoline, from broiled sardine, cooled beef, and beef extract. *Carcinogenesis* 5: 921.
- Okamoto, T., Shudo, K., Hashimoto, Y., Kosuge, T., Sugimura, T., and Nishimura, S. 1981. Identification of a reactive metabolite of the mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. *Chem. Pharm. Bull.* 29: 590.
- Överik, E., Nilsson, L., Fredholm, L., Levin, Ö., Nord, C.-E., and Gustafsson, J.-A. 1984. High mutagenic activity formed in pan-broiled pork. *Mutat. Res.* 135:149.
- Pariza, M.W., Ashoor, S.H., and Chu, F.S. 1979a. Mutagens in heat-processed meat, bakery and cereal products. *Food Cosmet. Toxicol.* 17: 429.
- Pariza, M.W., Ashoor, S.H., Chu, F.S., and Lund, D.B. 1979b. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Canc. Lett.* 7: 63.
- Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C., and Wasserman, A.E. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. *J. Food Sci.* 39: 314.
- Rappaport, S.M., McCartney M.C., and Wei, E.T. 1979. Volatilization of mutagens from beef during cooking. *Canc. Lett.* 8: 139.
- Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R. 1983. Interactions between the active metabolite of tryptophan pyrolysate mutagens, N-hydroxy-Trp-P-2, and lipids: The role of lipid peroxides in the commission of N-hydroxy-Trp-P-2 to non-reactive forms. *Chem.-Biol. Interactions* 45: 295.
- Shibamoto, T., Nishimura, O., and Mihara, S. 1981. Mutagenicity of products obtained from a maltol-ammonia browning model system. *J. Agric. Food Chem.* 29: 643.
- Singer, B. and Kusmierek, J.T. 1982. Chemical mutagenesis. *Ann. Rev. Biochem.* 52: 655.
- Sjödín, P., and Jägerstad, M. 1984. A balance study of <sup>14</sup>C-labelled 3H-imidazo[4,5-f]quinolin-2-amines (IQ and MeIQ) in rats. *Fd. Chem. Toxicol.* 22: 207.
- Spingarn, N.E. and Garvie, C.T. 1979. Formation of mutagens in sugar-ammonia model systems. *J. Agric. Food Chem.* 27: 1319.
- Spingarn, N.E. and Weisburger, J.H. 1979. Formation of mutagens in cooked foods. I. *Beef. Canc. Lett.* 7: 259.
- Spingarn, N.E., Garvie-Gould, C., Vuolo, L.L., and Weisburger, J.H. 1981. Formation of mutagens in cooked foods. IV. Effect of fat content in fried beef patties. *Canc. Lett.* 12: 93.
- Straus, D.S. 1981. Somatic mutation, cellular differentiation, and cancer causation. *J. Nat. Canc. Inst.* 67: 233.
- Sugimura, T., Kawachi, T., Nagao, M., Yahagi, T., Seino, Y., Okamoto, T., Shudo, K., Kosuge, T., Tsuji, K., Wakabayashi, K., Iitaka, Y., and Itai, A. 1977. Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. *Proc. Japan Acad.* 53: 58.
- Sugimura, T. 1978. Let's be scientific about the problem of mutagens in cooked food. *Mutat. Res.* 55: 149.
- Sugimura, T. 1982. Mutagens, carcinogens, and tumor promoters in our daily food. *Cancer* 49: 1970.
- Sugimura, T. and Sato, S. 1983. Mutagens-carcinogens in foods. *Canc. Res.* 43: 2415s.
- Takayama, S., Katoh, Y., Tanaka, M., Nagao, M., Wakabayashi, K., and Sugimura, T. 1977. In vitro transformation of hamster embryo cells with tryptophan pyrolysis products. *Proc. Japan Acad.* 53: 126.
- Takayama, S., Hirakawa, T., Tanaka, M., Kawachi, T., and Sugimura, T. 1979. In vitro transformation of hamster embryo cells with a glutamic acid pyrolysis product. *Toxicol. Lett.* 4: 281.
- Takayama, S., and Tanaka, M. 1983. Mutagenesis of amino acid pyrolysis products in Chinese hamster V79 cells. *Toxicol. Lett.* 17: 23.
- Takayama, S., Masuda, M., Mogami, M., Ohgaki, H., Sato, S., and Sugimura, T. 1984a. Induction of cancers in the intestine, liver, and various other organs of rats by feeding mutagens from glutamic acid pyrolysate. *Gann* 75: 207.
- Takayama, S., Nakatsuru, Y., Masuda, M., Ohgaki, H., Sato, S., and Sugimura, T. 1984b. Demonstration of carcinogenicity in F344 rats of 2-amino-3-methylimidazo[4,5-f]quinoline from broiled sardine, fried beef, and beef extract. *Gann* 75: 467.
- Tamano, S., Tsuda, H., Tatematsu, M., Hasegawa, R., Imaida, K., and Ito, N. 1981. Induction of  $\gamma$ -glutamyl transpeptidase positive foci in rat liver by pyrolysis products of amino acids. *Gann* 72: 745.
- Taylor, R.T., Fultz, E., and Shore, V. 1981. Food mutagen formation in model boiling systems. *Environ. Mutagen.* 3: 349.
- Taylor, R.T., Fultz, E., and Shore, V. 1984. Mutagen formation in a model beef boiling system. I. Conditions with a soluble beef-derived fraction. *J. Environ. Sci. Health* A19: 791.
- Taylor, S.L., Berg, C.M., Shoptaugh, N.H., and Scott, V.N. 1982. Lack of mutagens in deep-fat fried foods obtained at the retail level. *Food. Chem. Toxic.* 20: 209.
- Thompson, L.H., Carrano, A.V., Salazar, E., Felton, J.S., and Hatch, F.T. 1983. Comparative genotoxic effects of the cooked-food-related mutagens Trp-P-2 and IQ in bacteria and cultured mammalian cells. *Mutat. Res.* 117-243.
- Tohda, H., Oikawa, A., Kawachi, T., and Sugimura, T. 1980. Induction of sister-chromatid exchanges by mutagens from amino acid and protein pyrolysates. *Mutat. Res.* 77: 65.
- Tsuda, M., Takahashi, Y., Nagao, M., Hirayama, T., and Sugimura, T. 1980. Inactivation of mutagens from pyrolysates of tryptophan and glutamic acid by nitrite in acidic solution. *Mutat. Res.* 78: 331.
- Tsuda, M., Nago, M., Hirayama, T., and Sugimura, T. 1981. Nitrate converts 2-amino- $\alpha$ -carboline, an indirect mutagen, into 2-hydroxy- $\alpha$ -carboline, a non-mutagen and 2-hydroxy-3-nitroso- $\alpha$ -carboline, a direct mutagen. *Mutat. Res.* 83: 61.
- Tsuda, M., Wakabayashi, K., Hirayama, T., Kawachi, T., and Sugimura, T. 1983. Inactivation of potent pyrolysate mutagens by chlorinated tap water. *Mutat. Res.* 119: 27.
- Vithayathil, A.J., Commoner, B., Nair, S., and Madyastha, P. 1978. Isolation of mutagens from bacterial nutrients containing beef extract. *J. Toxicol. Environ. Health* 4: 189.
- Wakabayashi, K., Tsuji, K., Kosuge, T., Takeda, K., Yamaguchi, K., Shudo, K., Iitaka, Y., Okamoto, T., Yahagi, T., Nagao, M., and Sugimura, T. 1978. Isolation and structure determination of a mutagenic substance in L-lysine pyrolysate. *Proc. Japan Acad.* 54: 569.
- Wang, Y.Y., Vuolo, L.L., Spingarn, N.E., and Weisburger, J.H. 1982. Formation of mutagens in cooked foods. V. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. *Canc. Lett.* 16: 179.
- Watanabe, J., Kawajiri, K., Yonekawa, H., Nagao, M., and Tagashira, Y. 1982. Immunological analysis of the roles of two major types of cytochrome P-450 in mutagenesis of compounds isolated from pyrolysates. *Biochem. Biophys. Res. Comm.* 104: 193.
- Weisburger, J.H. and Williams, G.M. 1980. Chemical Carcinogens. In "Toxicology: The Basic Science of Poisons," ed. J. Doull, C. Klassen, and M. Amdur, p. 84. Macmillan, New York.
- Wynder, E.L., and Gori, G.B. 1977. Contribution of the environment to cancer incidence: an epidemiologic exercise. *J. Nat. Canc. Inst.* 58: 825.
- Yamada, M., Tsuda, M., Nagao, M., Mori, M., and Sugimura, T. 1979. Degradation of mutagens from pyrolysates of tryptophan, glutamic acid and globulin by myeloperox-

- idase. *Biochem. Biophys. Res. Comm.* 90: 769.
- Yamaguchi, K., Zenda, H., Shudo, K., Kosuge, T., Okamoto, T., and Sugimura, T. 1979. Presence of 2-amino-dipyrido[1,2-a:3',2'-d]imidazole in casein pyrolysate. *Gann* 70: 849.
- Yamaguchi, K., Shudo, K., Okamoto, T., Sugimura, T., and Kosuge, T. 1980. Presence of 2-amino-dipyrido[1,2-a:3',2'-d]imidazole in ordinary broiled cuttlefish. *Gann* 71: 743.
- Yamaguchi, T. and Nakagawa, K. 1983. Reduction of induced mutability with xanthine and imidazole-derivatives through inhibition of metabolic activation. *Agric. Biol. Chem.* 47: 1673.
- Yamaizumi, A., Shiomi, T., Kasai, H., Wabayashi, K., Nagao, M., Sugimura, T., and Nishimura, S. 1980. Quantitative analysis of a novel potent mutagen, 2-amino-3-methyl-imidazo[4,5-f]quinoline, present in broiled food by GC/MS. *Koenshu-Iyo Masu Kenkuyakai* 5: 245.
- Yamamoto, T., Tsuji, K., Kosuge, T., Okamoto, T., Shudo, K., Takeda, K., Iitaka, Y., Yamaguchi, K., Seino, Y., Yahagi, T., Nagao, M., and Sugimura, T. 1978. Isolation and structure determination of mutagenic substances in L-glutamic acid pyrolysate. *Proc. Japan Acad.* 54: 248.
- Yamazoe, Y., Ishii, K., Kamataki, T., Kato, R., and Sugimura, T., 1980a. Isolation and characterization of active metabolites of tryptophan pyrolysate mutagen, Trp-P-2, formed by rat liver microsomes. *Chem. Biol. Interact.* 30: 125.
- Yamazoe, Y., Yamaguchi, N., Kamataki, T., and Kato, R. 1980b. Metabolic activation of Trp-P-2, a mutagenic amine from tryptophan-pyrolysate, by liver microsomes from 3-methylcholanthrene-responsive and non-responsive mice. *Xenobiotica* 10: 483.
- Yokota, M., Narita, K., Kosuge, T., Wabayashi, K., Nagao, M., Sugimura, T., Yamaguchi, K., Shudo, K., Iitaka, Y., and Okamoto, T. 1981. A potent mutagen isolated from a pyrolysate of L-ornithine. *Chem. Pharm. Bull.* 29: 1473.
- Yokoyama, S., Miyazawa, T., Kasai, H., Nishimura, S., Sugimura, T., and Iitaka, Y. 1980. Crystal and molecular structures of 2-amino-3-methyl-imidazo[4,5-f]quinoline, a novel potent mutagen found in broiled food. *FEBS Lett.* 122: 261.
- Yoshida, D., and Matsumoto, T. 1978. Changes in mutagenicity of protein pyrolysates by reaction with nitrite. *Mutat. Res.* 58: 35.
- Yoshida, D., Matsumoto, T., Yoshimura, R., and Matsuzaki, T. 1978. Mutagenicity of amino- $\alpha$ -carbolines in pyrolysis products of soybean globulin. *Biochem. Biophys. Res. Comm.* 83: 915.
- Yoshida, D., and Okamoto, H. 1980a. Formation of mutagens by heating creatine and glucose. *Biochem. Biophys. Res. Comm.* 96: 844.
- Yoshida, D., and Okamoto, H. 1980b. Formation of mutagens by heating the aqueous solution of amino acids and some nitrogenous compounds with addition of glucose. *Agric. Biol. Chem.* 44: 2521.
- Yoshida, D., and Okamoto, H. 1982. Mutagenicity of the pyrolysis products of ammonium salts. *Agric. Biol. Chem.* 46: 1067.
- Yoshida, D., and Fukuhara, Y. 1982. Formation of mutagens by heating creatine and amino acids. *Agric. Biol. Chem.* 46: 1069.
- Yoshida, D., Saito, Y., and Mizusaki, S. 1984. Isolation of 2-amino-3-methyl-imidazo[4,5-f]quinoline as mutagen from the heated product of a mixture of creatinine and proline. *Agric. Biol. Chem.* 48: 241.

---

Based on a paper presented during the IFT Muscle Foods Division Program, "Muscle Food Consumption in Relation to Human Health: Concerns and Controversies," at the 44th Annual Meeting of the Institute of Food Technologists, Anaheim, Calif., June 10-13, 1984.

---