CHAPTER II

REVIEW OF LITERATURE

Gastric Cancer

Gastric cancer is declining in incidence but still remains an important cause of mortality. The causes of gastric cancer are unknown, but genetics, environmental, infective, and premalignant factors have been implicated. There are two distinct phases to gastric carcinogenesis. Firstly, gastric atrophy develops and results in decreased gastric acidity, bacterial colonisation of the stomach and the reduction by bacteria of dietary nitrate (present in water, vegetables, and cured meats) to nitrite. In second phase the nitrite reacts in vivo with certain protein substrates (amines, amides, and ureas) to form carcinogenic N-nitroso compounds (Correa et al., 1985; Souhami and Tobias, 1995). These are carcinogens in animals (causing intestinal metaplasia of the gastric mucosa in rats), and may be so in man. A hypothesis for the stepwise causation of gastric carcinoma is shown in Figure 2.1 (Souhami and Tobias, 1995).

A case-control study of diet and stomach cancer was conducted in two hundred forty-six histologically verified cancer cases, which individually matched by age, sex, and area of residence to 246 randomly selected population controls. Daily

nutrient consumption values questionnaire data through the use of the US Department of Agriculture Food Composition Data Bank were extended and modified for Canadian items. It was found that increasing nitrate/nitrite intake was associated with increases risk of gastric cancer (Risch et al., 1985).

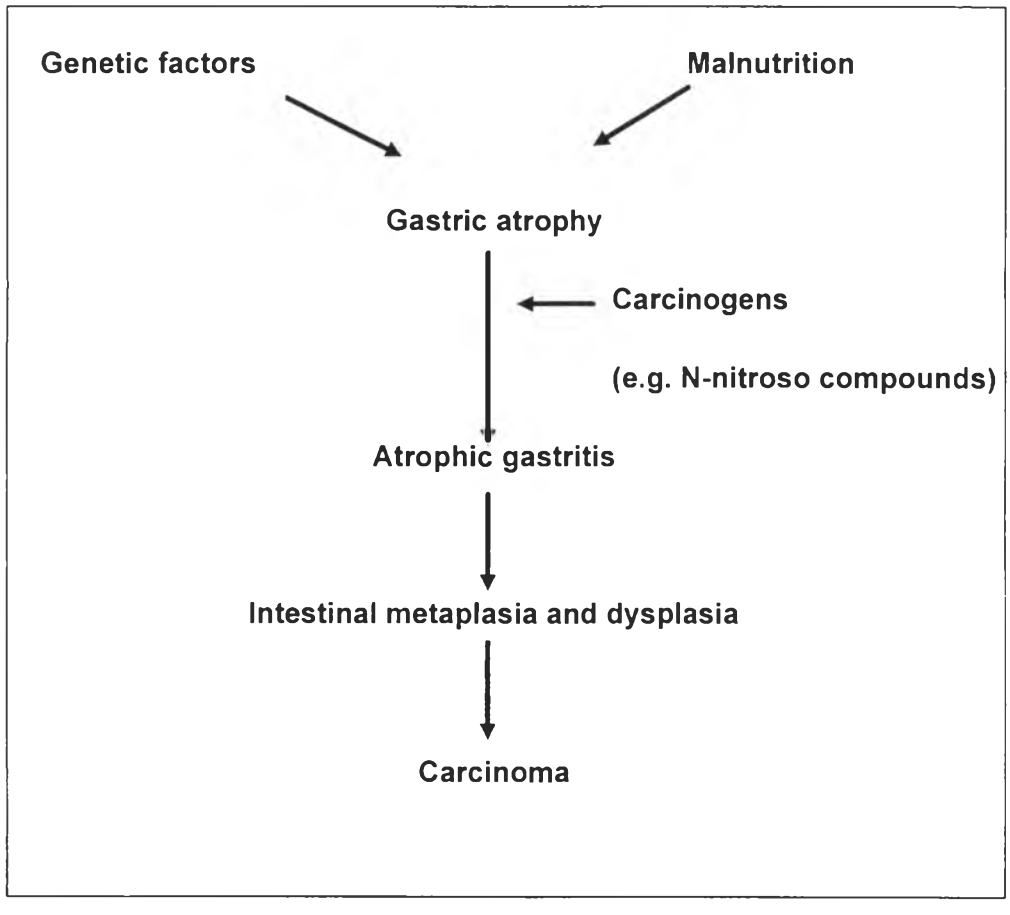


Figure 2.1 A hypothesis for the causation of gastric cancer.

An epidemiological study has revealed that areas with high nitrate in drinking water have an increased death rate from gastric cancer, compared with low nitrate control areas (Hill, Hawksworth, and Tattersall, 1973). It is certain that reduction in the amount of nitrite to cured meat or food additive would lead to a

reduction in amount of N-nitroso compounds in food when sold and consumed. The additional advantage that less nitrite would be present to the stomach with nitrosable is further reason to recommend reduction in the amount of nitrite used in meat processing; however, many manufacturers continue to use the large amounts of nitrite.

Nitrite

Nitrite is used as a preservative in cured meat product and cheese including hot dog, bacon, ham, lunch meat, and smoked fish. Adding nitrite to foodstuffs, it performs the following functions: (a) it produces the characteristic cured meat color and affects flavor; (b) it has antioxidant activities which prevent the warmed-over flavor; and (c) it retards *Clostridium botulinum* growth and toxin production, which can occur if the product is mishandled and temperature-abused (Swann, 1977; Sofos, Busta, and Allen, 1979; Fiala et al., 1985). Botulism is a rare but very often fatal neuroparalytic disease affecting man and animals (Sofos et al., 1979).

Exposure to Nitrite Nitrate and nitrite are common constituents of the normal diet. The nitrate ion is essential for plant growth and occurs in soil either naturally or added in the form of fertilizer. Soil-derived nitrate may be consumed by man in the form of plant material, especially in those concentrating nitrate such as

celery, spinach and beet, and from drinking water. In man nitrate is absorbed from the small intestine, it is secreted in the saliva and gastric juice and excreted in urine. It is readily reduced to nitrite by bacterial action both during storage of nitrate-containing food, especially when the food is not refrigerated, and within oral cavity, or the intestine in human body (Fairweather, 1981).

The acidic conditions of the stomach are suitable for nitrosation by nitrite from food and swallowed saliva. About 25% of ingested nitrate is recirculated into the saliva of which about 20% (5% of the ingested nitrate) is then reduced to nitrite by oral bacteria. Of the nitrite entering the stomach, about 20% arises directly from food and about 80% from the reduction of salivary nitrate (Challis, 1985). The maximum normal dietary intake of nitrite is in the order of 12 mg per day consisting of about 3 mg per day of nitrite in food and 9 mg per day salivary nitrite (Gatehouse and Tweats, 1982).

Toxicity of Nitrite Nitrate is not potentially harmful unless it is converted to nitrite. Nitrite is considerably more toxic. It has two pricipal effects : relaxation of vascular and other smooth muscles and formation of methemoglobin. In lower doses the symptoms are flushing of the face and extremities, gastrointestinal discomfort, and headache; in larger toxic doses cyanosis, nausea, vomiting, abdominal pain, and collapse occur. The lower stomach acidity of infants under four months of age may permit growth of bacteria capable of reducing nitrate to nitrite. When water with high

nitrate content was given to young infants, deaths have resulted (Fassett, 1973). Poisoning of adults by nitrite has not been a problem, except when large amounts of nitrite were mistakenly added to foods sensitive individuals may develope severe headaches ("hot dog headaches") after eating such cured meats as frankfurter, bacon, salami, and ham (Henderson and Raskin, 1972).

Nitrite presents a toxic hazard to man due to (1) direct toxicity of nitrite (methemoglobinaemia in children), (2) formation of N-nitroso compounds (nitrosamines and nitrosamides) following reaction between nitrite and secondary tertiary amines and amides (Fairweather, 1981). The exact lethal dose in humans is not known but is estimated to be about 1 g in adults. The acceptable daily intake for nitrite suggested by the World Health Organization (WHO) is 5 mg per kg of food in addition to naturally occurring nitrate, and that for nitrite 0.4 mg per kg of food (Fassett. 1973).

Nitrosable precursors of mutagens Exposure to nitrate and nitrite, precursors of carcinogenic N-nitroso compounds, have also been correlated with stomach cancer mortality (Mirvish, 1983). Dialkylamines and trialkylamines are well-known precursors for the N-nitrosamines in foods. These amines are generally found in fish meats and they can undergo nitrosation by reaction with nitrite under gastric conditions to form carcinogenic dialkylnitrosamines (Mirvish, 1975).

Several nitrosable mutagen precursors have been isolated and their structures have been determined. Some nitrosatable precursors found are aromatic or heterocyclic compounds rather than alkylnitrosamides. Therefore, this can classified nitrosable mutagen precursors following:

N-nitroso Compounds N-nitroso compounds are the most broadly acting group of carcinogens and they include some of the most potent carcinogens. Because they are most effective when administered in small frequent dose over a long period, human exposure to even small amounts of nitrosamines, if frequent, could contribute to development of cancer (Lijinsky, 1977).

N-nitroso compounds are usually formed by the interaction of a nitrosating agents, i.e. nitrous anhydride (N_2O_3), nitrous acidium ion ($ON\text{-}OH_2^+$), and nitrosonium ion (NO^+), derived from either nitrite salts or nitrous acid with an amino substance under acidic conditions as shown in Figure 2.2 (Kikugawa and Kato, 1991).

The amino substance can be one of several types of compound such as secondary and tertiary amines, secondary and tertiary amides, peptides, substituted ureas, guanidines and urethanes. In the case of amines, amides and ureas, the products are the N-nitrosamines, N-nitrosamides and N-nitrosoureas shown in Figure 2.3. The mechanism and extent of formation of the N-nitroso derivative is dependent

on both the structure of the amino substance and the source of the nitrosating agent, as well as the reaction condition (Challis, 1985).

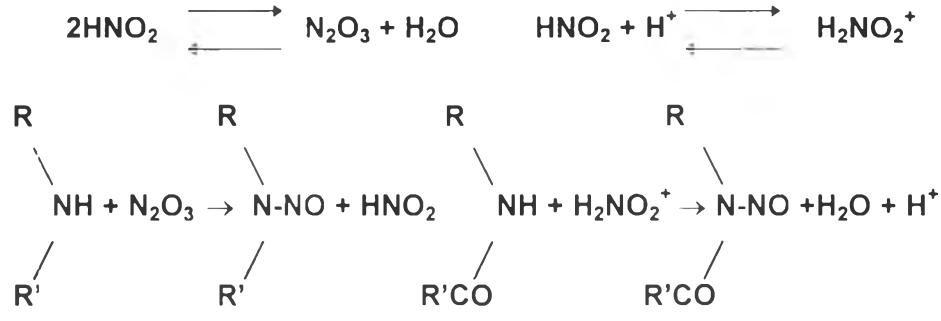


Figure 2.2 Formation of nitrosamines and nitrosamides under acidic conditions.

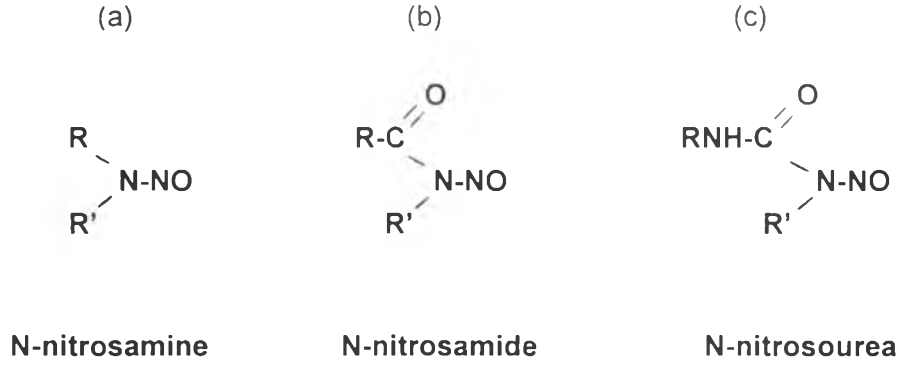


Figure 2.3 N-nitroso compounds formed from (a) amines, (b) amides and (c) ureas. R, R' = alkyl, aryl or part of a cyclic structure.

N-nitroso compounds can be divided into two classes according to their action as carcinogens.

1) Nitrosamines act indirectly, requiring metabolic activation.

2) Nitrosamides act directly, are considered not to need metabolic activation

Carcinogenic N-nitroso compounds are not commonly found in the environment, and then only in trace amounts (levels of a few parts per billion). However, they are readily formed by reaction of secondary or tertiary amines with nitrites in a wide range of conditions, but the optimal pH is near 3, and especially in the acidic medium of the mammalian stomach (Lijinsky, 1977). The major part of man's exposure to nitroso compounds comes from compounds synthesized in his own stomach by the reaction between dietary amines and the nitrite in his saliva (Swann, 1977).

Indeed, dimethylnitrosamine has been found in several meats and fish, as has nitrosoproline, formed by reaction of proline with nitrite. Even through nitrosoproline is itself not carcinogenic, it is converted by heating into nitrosopyrrolidine, which is carcinogenic (Lijinsky, 1977). The nitrosation of thioproline is very rapid, and N-nitrosothioproline is nonmutagenic. It may act by

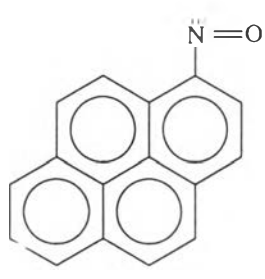
blocking the formation of carcinogenic or mutagenic N-nitroso or diazo compounds by trapping nitrite and then being excreted in the urine (Kikugawa and Nagao, 1991).

Non N-nitroso Compounds Several research groups looked for the presence of nitrosable mutagen precursors in foods, to produce direct-acting mutagen by reaction with nitrite. Japan also had a high incidence of stomach cancer. Japanese fish, soy sauce, bean paste, fish sauce, and Chinese cabbage showed direct-acting mutagenicity on *Salmonella typhimurium* TA 100 after treatment with nitrite. The precursors of the mutagens were isolated and identified as nitrosated products of indole derivatives, aromatic compounds (Marquardt, Rufino, and Weisburger, 1977; Wakabayashi et al., 1983; 1985).

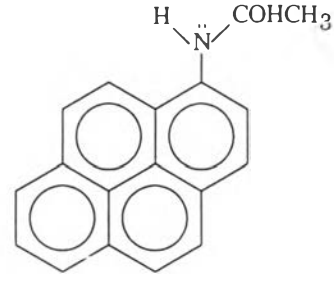
Heterocyclic amines, such as aminoimidazo-quinoline, aminoimidazo-quinoxaline, and aminoimidazo-pyridine compounds, which are found in cooked foods and are mutagens to bacteria with S9 mix before nitrite treatment, became mutagenic to TA 98, TA 100, and *E. coli* WP2 *uvrA* without S9 mix following treatment with 50 mM nitrite (Sasagawa, Muramatsu, and Matsushima, 1988). Formation of a nitro derivative by nitrite treatment was observed with mutagenic and carcinogenic 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ). IQ was converted to 3-methyl-2-nitroimidazo[4,5-f]quinoline, showing mutagenicity towards *Salmonella* strains without S9 mix, after 50 mM nitrite treatment at pH 3 (Tsuda et al., 1985).

Aminopyrene

Aminopyrene (1-aminopyrene) is a metabolite produced during the incubation of 1-nitrosopyrene in the suspension cultures of Chinese hamster ovary cells (Heflich, Fulierton, and Beland, 1986). The chemical structures of 1-aminopyrene and 1-nitrosopyrene are shown in Figure 2.4 (Edwards et al., 1986).



1-nitrosopyrene



1-aminopyrene

Figure 2.4 Structure of 1-nitrosopyrene and 1-aminopyrene

Aminopyrene itself is a weak mutagen; however, Manoonphol (1994) found that after the incubation with nitrite in gastric like condition (Table 2.1) the mutagenicity of the final reaction mixture was much stronger (31-69 times of the untreated aminopyrene on TA 98 and 5.5-7.8 times of the untreated compound on TA 100).

Table 2.1 Mutagenicity of aminopyrene treated with nitrite (500 mM) in gastric like condition on *Salmonella typhimurium* TA 98 and TA 100 (Manoonphol,1994).

Chemical	Amount	TA 98		TA 100	
	(μg/plate)	without $NaNO_2$	with $NaNO_2$	without $NaNO_2$	with $NaNO_2$
aminopyrene	0	12±3	22±4	118±28	126±24
	0.06	19±5	595±73	92±14	ND
	0.12	26±11	1814±50	106±23	584±78
	0.24	80±10	ND	132±28	1027±101

Results are shown as means ± SD (n=14).

A solution of 1-aminopyrene in dimethyl sulfoxide exposed to an artificial source of near ultraviolet light (600 kJ/m^2) induced significant direct-acting utilizing strain TA 98. The high-performance liquid chromatography of this solution resulted in a fraction that was mutagenic on TA 98 but inactive on a nitroreductase-deficient strain of *Salmonella* (TA 98 NR). This observation suggested the presence of a nitro-containing compound as a product of irradiation the 1-aminopyrene (Okinaka et al., 1986).

Studied in Chinese hamster lung cell, 1-aminopyrene (0.25-25 μg/ml) were cytotoxic, and induced the formation of DNA lesions, which were measured as DNA single-strand breaks after sedimentation in alkaline sucrose-density gradients. Higher doses of 1-aminopyrene (25-60 μg/ml) inhibited the formation of DNA single-strand breaks (Edwards et al, 1986).

One-aminopyrene was treated with sodium nitrite at pH 3 and 37 ^{o}C for 4 h. The reaction mixture of 1-aminopyrene showed mutagenicity to *Salmonella typhimurium* TA 98 and TA 100 strains without metabolic activation. The numbers of His^{+} revertant colonies to TA 98 strain were 870/0.02 μM 1-aminopyrene. The mutagens from 1-aminopyrene were unidentified nitro-introduced compound and 1-nitropyrene (Kato et al, 1991).

Dietary Fiber

Dietary fiber is composed of a number of complex and highly variable chemical compounds each with a unique chemical and structure arrangement which imparts its specific physicochemical, functional, and nutritional properties. These components are dynamic and undergo numerous changes during the growth and maturation of plant tissues and during the processing, storage, and cooking of foodstuffs (Dreher, 1987a). The original concept of dietary fiber focused on these components derived from cell walls. However, this view has expanded after much

controversy to include "the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the human gastrointestinal tract" (Dreher, 1987b, Quoting Trowell, 1985).

The most abundant compounds identified as fiber are in the plant cell wall. Some of the compounds are part of the intracellular cement. Others are secreted by the plant in response to injury, and others prevent seeds from desicating. Based on its intra-plant functions, dietary fiber is divided into three major fractions summarized in Table 2.2 (Schneeman, 1986; Dreher, 1987a).

A Possible Antagonist of Nitrite Moller et al. (1988) found that wheat bran acted as a nitrite scavenger under conditions similar to those that exist in the stomach. Fiber of fruits and vegetables, such as cabbage, chinese kale, pomelo peels, cucumber, waxgourd, ivy gourd, papaya, rice bran, and pineapple core, had different nitrite scavenging capability which were ranging from 0.1 to 0.25 μg nitrite per mg fiber. It was clearly demonstrated that home cooking or the ripening of fruits and vegetables did not significantly affect on nitrite scavenging capability (Laohavechvanich, 1994).

Table 2.2 Classification of major dietary fiber components.

Component	Main chemical structure	Main variation
A. Structural polysaccharides and derivatives		
1.Cellulosic polysaccharides		
a. Cellulose	β (1,4)glucan	DP*
b. Cellulose derivatives	β (1,4)glucan	carboxymethyl, hydroxypropyl and methyl; DP*, DS**, and MS***
2. Noncellulosic polysaccharides		
a. Hemicellulose	β-xylans; β-mannans; β-arabinogalactans; β-arabinans	number and distribution of side chain
b. Pectin	α-galacturonans	methoxy groups; number and distribution of side chains; type of cation
c. Gums isolated from plant cell walls		
Carrageenan	(1,4)(1,3) linked sulfated galactans	type of linkage; degree of sulfation; cation
Alginic acid	linear copolymer	ratio of mannuronic/ guluronic acids

Table 2.2 (continue) Classification of major dietary fiber components.

Component	Main chemical structure	Main variation
B. Structural nonpolysaccharide components		
Lignin	phenylpropane polymers	DP*; functional group
C. Nonstructural polysaccharide components		
1. Mucilages (e.g. guar and locust bean gum)	β (1,4)galactomannans	ratio and distribution of side chains
2. Exudates (e.g.gums: arabic, ghatti, karaya)	heteropolysaccharides	ratio of main chain components; number and distribution of side chains; methoxy groups; cations
3. Xanthan gum	heteropolysaccharides	-

* DP = degree of polymerisation

** DS = degree of substitution

*** MS = molar substitution

Adsorption of Mutagens Harris et al. (1991) indicated that cell wall from potato skins strongly adsorbed 1, 8 dinitropyrene (DNP). In marked contrast to the skin walls, potato flesh walls adsorbed only a small proportion of the DNP. The ability of three unlignified cell-wall preparations to adsorb DNP was studied by Ferguson et

al. (1992). It was suggested that the greatest adsorption occurred with walls from leaf blade, followed by petiole and corm walls, although the differences were not major. The amount of adsorption was intermediate between the low adsorption previously found with unlignified dicotyledon walls (from the flesh of potato tubes and immature cabbage leaves) and the much higher adsorption found with unlignified walls from monocotyledons of the grass and cereal family (from leaves of seeding Italian ryegrass).

Takeuchi et al. (1988) found that refined corn bran effectively adsorbed DNP. The adsorption of DNP to the fibers appeared irreversible since little mutagenicity was recovered by washing the treated fibers with aqueous buffer solution of various pHs. Refined corn bran could similarly adsorb mutagenic heterocyclic amines such as IQ, Trp-P-1, Trp-P-2, Glu-P-1, and Glu-P-2. Laohavechvanich (1994) found that the fiber of fruits and vegetables had antimutagen formation of aminopyrene treated with nitrite. The mutagenicity was reduced with a dose-response relationship.

Food Polysaccharides (Fibers)

Adsorption Properties Adsorption of organic molecules includes bile acids, cholesterol, and toxic compounds. In vitro studies have demonstrated that lignin is a potent bile acid adsorbent. Pectin and other acidic polysaccharides also

seem to sequester bile acids. Cellulose, in contrast, has little bile-acid binding ability. The ability to increase fecal bile acid excretion has been correlated to the plasma cholesterol lowering effect of certain soluble, noncellulose polysaccharides such as pectin and guar gum (Schneeman, 1986).

Wheat bran, guar gum, Konjac mannan (a glucomannan), chitosan, and isolated lignin have been shown to bind bile acids in the small intestinal contents. In humans, pectin, guar gum, oat bran, and wheat bran have been shown to increase fecal bile acid excretion (Schneeman and Tietyen, 1994). Binding of vitamin E by lupin and gum were higher ($p \leq 0.05$) at pH 2 and pH 7 than at the sequential treatment. Affinities for vitamin E were : sugar beet > oat > lupin > barley > gum. Capacities were : barley > gum > lupin > sugar beet > oat. Vitamin E binding to dietary fiber is complex, multifactorial, and not due to a single mechanism (Nnanna and O'Neill, 1992).

Cation Exchange Capacities Acidic polysaccharides with uronic acid moieties can act as cation exchangers, i.e. can bind metals. Alginic acid's affinity for cations depends on the source and relative amounts of the acidic sugars, mannuronic acid and guluronic acid. Guluronic acid-rich alginates have a higher affinity for divalent ions than mannuronic acid-rich samples. The cation exchange properties are mainly related to free carboxyl groups in pectin and to a lesser extent in hemicelluloses (Eastwood, 1973). Casterline and Ku (1993) suggested that

binding capacities of commercially available individual fiber components decreased in the order : lignin > polypectin > pectin > gum > cellulose. Zinc-binding capacities of various dietary fiber types differ, accounting for different zinc bioavailabilities of some foods.

Pectin shows the capacity to bind divalent mineral elements, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, and Zn^{2+}. This behavior seems to be due to interactions, either from electrostatic forces or from complex formation, between the cation and the carboxylic functional group of the D-galacturonic acid units in the pectin. For this reason, pectins with a high degree of esterification are shown to bind significantly less amounts of minerals than ones with a low degree of esterification. It was suggested that Ca^{2+} and Zn^{2+} ions were bound with intramolecular eletrostatic bonds, whereas Cu^{2+} formed complexes (Torre, Rodriguez, and Saura-Calixto, 1991). On the contrary, Platt and Clydesdale (1987) reported that the mineral-binding capacity of both high and low degree of esterification pectin may be due to their physical structure rather than to the amount of available carboxyl groups of this polymer. In effect, pectins with a high degree of esterification formed very elastic gels, which allows water and solute to distribute interstitially and on the surface of the gels, while solute mobility is esterically hampered in pectins with a low degree of esterification.

Nair et al. (1987) reported that mineral binding to guar gum was negligible, while sterculia gum bound Zn^{2+} and Cu^{2+} in amount that were proportional to the

concentration of fiber in the study. This behavior is attributed to complex formation between the metallic element and the carboxylic groups in the polysaccharide (1% uronic acid in guar gum and 50% in sterculia gum). This interpretation agrees with the data of Ha et al. (1989), who studied calcium binding to anionic (alginate, karaya, arabic, and ghatti) and neutral (locust bean and guar) gums. Anionic gums showed higher calcium-binding affinity than neutral gums. Alginate seemed to have the highest affinity for calcium among the gums studied. However, the evidence showed that the order of magnitude of uronic acids in each gum did not agree with the order of calcium-binding affinity, indicating limited importance of the chemical nature of hydrocolloids to the effect of linear charge density.

Salmonella Mutagenicity Test

The literature on the *Salmonella* test has grown enormously since the publication of the original methods paper, and test data on more than 5,000 chemicals have been published (Maron and Ames, 1983, quoting Environmental Mutagen Information Center Index, 1982). Many of the mutagenic components of these mixtures have been characterized chemically. A considerable number of mutagens first detected by the *Salmonella* test have been shown subsequently to be carcinogenic in animal test (Ohgake et al., 1982).

The *Salmonella* test was first validated in a study of 300 chemicals, most of which were known carcinogens (McCann et al., 1975; McCann and Ames, 1976). It was subsequently validated in studies by the Imperial Chemical Industries (Purchase et al., 1976), the National Cancer Center Research Institute in Tokyo (Sugimura et al., 1976), and the International Agency for Research on Cancer (Bartsch et al., 1980). Nearly 90% of the carcinogens tested were mutagenic in their studies, but there was considerable overlapping of chemicals tested. Ames and McCann (1981) estimated the correlation to be about 83%. All the validations show that the test fails to detect a few classes of carcinogens such as polychlorinated pesticides (Rinkus and Legator, 1979, 1981; Ames and McCann, 1981).

The Bacterial Tester Strains The mutagenicity testing used the histidine- requiring strains. Each tester strain contains a different type of mutation in the histidine operon (Table 2.3). In addition to the histidine mutation, the standard tester strains contain other mutations that greatly increase their ability to detect mutagens. One mutation (*rfa*) causes partial loss of the lipopolysaccharide barrier that coats the surface of the bacteria and increases permeability to large molecules such as benzo[a]pyrene that do not penetrate the normal cell wall. The other mutation (*uvrB*) is a deletion of a gene coding for the DNA excision repair system, resulting in greatly increased sensitivity in detecting many mutagens (Ames, Lee, and Durtston, 1973a).

For technical reasons, the deletion excising the *uvrB* gene extends through the *bio* gene and as a consequence, these bacteria also require biotin for growth. The standard tester strains, TA 97, TA 98, TA 100, and TA 102 contain the R-factor plasmid, pKM101. TA 100 detect mutagens that cause base-pair substitutions, primarily at one of G-C pairs. The *hisD3052* mutation in TA 98 is in the *hisD* gene coding for histidinol dehydrogenase. TA 98 detect various frameshift mutagens. Frameshift mutagens can stabilize the shifted pairing that often occur in repetitive sequences or 'hot spots' of the DNA, resulting in a frameshift mutation which restores the correct reading frame for histidine synthesis (Isono and Yourno, 1974).

Nitroreductase-deficient derivatives of TA 100, and TA 98 were isolated by Rosenkranz and Speck (1975; 1976). These are useful for studying the metabolism and mutagenicity of nitro carcinogens such as nitrofurazone and furylfuramide which are activated directly to mutagens by bacterial nitroreductases. Rosenkranz and Speck (1975; 1976) used nitroreductase-deficient strains to demonstrate the mutagenic activation of some nitro-containing therapeutic agents and showed that mammalian liver nitroreductases can also activate this class of carcinogens. Nitrated pyrenes are extremely potent mutagens (Rosenkranz et al., 1980).

Table 2.3 Genotypes of the TA strains used for mutagenesis testing

Histidine mutation				LPS	Repair	R-factor
hisD6610 his01242 = TA 88	**hisD3052**	**hisG46**	**hisG428 (pAQ1)**			
TA 90	TA 1538	TA 1535	-	*rfa*	Δ*uvrB*	-R
[TA 97]	[TA 98]	[TA 100]	-	*rfa*	Δ*uvrB*	+R
-	TA 1978	TA 1975	-	*rfa*	+	-R
TA 110	TA 94	TA 92	-	+	+	+R
-	TA 1534	TA 1950	-	+	Δ*uvrB*	-R
-	-	TA 2410	-	+	Δ*uvrB*	+R
TA 89	TA 1964	TA 1530	-	Δ*gal*	Δ*uvrB*	-R
-	TA 2641	TA 2631	-	Δ*gal*	Δ*uvrB*	+R
-	-	-	[TA 102]	*rfa*	+	+R

Tester strains in brackets are recommended for general mutagenesis testing. All strains were originally derived from *S. typhimurium* LT2. Wild-type genes are indicated by a +. The deletion (Δ) through *uvrB* also includes the nitrate reductase (*chl*) and biotin (*bio*) genes. The Δ*gal* strains and the *rfa/uvrB* strains have a single deletion through *gal chl bio uvrB*. The *rfa* repair$^+$ strains have a mutation in *galE*. R = pKM101. The tester strain TA 1536, included in the original tester set (Ames et al., 1973a), and all other strains containing the histidine mutation *hisC207* have been discontinued as they are reverted by only a few mutagens and these can be detected well by other tester strains. TA 97 replaces TA 1537 and TA 2637.