Chapter 1

INTRODUCTION

1.1 Opportunities for cancer prevention by diet.

It is well documented that certain mutagenic chemicals play a predominated role in the etiology of cancer (Ames, 1979; Ames,1989 a, Ames, et al., 1987). Furthermore, it has become evident that environmental agents, especially diets, are responsible for much of the incidences of cancer (National Research Cancer, 1982; Ames, 1983). Cancer is one of the degenerative diseases of old age, though exogenous factors (environmental carcinogens) can substantially increase it or decrease it. Epidemiologists have been accumulating evidence that unbalanced diets are major contributors to heart disease and cancer (Ames, et al., 1987). The main dietary imbalances are too few fruits and vegetables and too much fat. Particular micronutrients in fruits and vegetables are considered to be important in disease prevention (Ames, et al., 1987; Ames, 1989b). Micronutrients are components of the defenses against oxidants and other endogenous mutagens contributing to the degenerative diseases associated with aging, cancer, heart disease, etc. Recent investigations on the detection of natural and synthetic mutagenic factors by means of microbial assays revealed that a

number of environmental chemicals, especially have found in foods, may be adversely affecting human health (Sugimura and Sato, 1983). Identification of mutagenic factors in the environment is concerned because they may represent important health hazard to man. It has been important to examine the genotoxicity of chemicals in our environment particularly in our diet. Concurrent with this type of research, it may also be propitious to investigate if antimutagenic substances are present in our food. There are many mutagens and/or carcinogens in food (Furihata and Matsushima, 1986). The mutagens/carcinogens found so far include mycofoxins such as aflatoxin B_1 (AFB_1), nitrosamines and aromatic hydrocarbons (Miller and Miller, 1986). Recently, an intensive search for novel antimutagens/anticarcinogens from natural resources was undertaken, and various type of dietary inhibitor of mutagenesis /carcinogenesis such as β-carotene, tocopherol, vitamin C, and polyphenol have been isolated and identified (National Research Council,, 1982; Willett and MacMahon 1984).

As a consequence of research, there has been immense improvement in human health and longevity by prevention of the menace of infections and nutritionally-related diseases. It has been the dream of cancer research scientists to achieve comparable success in cancer

prevention (Wynder, 1977). Toward this goal, much has been accomplished by identifying and controlling human exposures to carcinogens. However, as the inevitability of cancer is recognized despite the best efforts at reducing carcinogenic exposures, the role of diet as one of the major determinants of cancer, has received renewed recognition in recent years. The impact of nutrition on cancer incidence of experimental animals was significant portion of the research in carcinogenesis. Nowadays, other favorable research on the role of diet on cancer incidence approaches to prevention of cancer. Dietary inhibitors of carcinogenesis fall into three groups, (Wattenberg, 1985) as the following :

1. The levels of the major dietary components, fat, carbohydrate and protein.
2. The known minor essential dietary components, such as vitamins and trace elements including selenium.
3. Factors that are not now considered to be essential dietary components but do inhibit carcinogenesis.

1.2 Mutagenesis, mitogenesis and carcinogenesis.

Geneticists have known that cell division is critical for mutagenesis, and mutagenesis may be important for carcinogenesis. The inactivation of tumor suppressor genes is also known to be important in carcinogenesis and recent evidence suggests that one of the functions of tumor suppressor genes is to inhibit mitogenesis (Stanbridge, 1990). Once the first copy of tumor suppressor gene is mutated, the inactivation of the second copy is dependent on cell division than by an independent second mutation (Ames and Gold, 1991). While the stimulation of mitogenesis increases the chance of every mutational step, it is a much more important factor for tumor induction after the first mutation has occurred. It can be explained that mutagenesis and mitogenesis are synergistic (Ames and Gold, 1991) and mitogenesis after the first mutation is more effective than before. The studies by on radiation has supported the idea that both mutagenesis and mitogenesis are important in tumor induction (Jones, et al., 1983; Little, et al., 1985).

1.3 Mutagenesis as an index of carcinogenesis

Mutagenesis is any alteration in the purine and pyrimidine sequence in DNA. If the alteration is compatible with life and replication of the cells, the mutation usually becomes a permanent part of the DNA and is inherited by all of the progeny of the cell.

Chemical mutagenesis usually results from reaction of an electrophilic chemical or its metabolite with one of the purine or pyrimidine bases in the DNA to form an adduct. The adduct may cause a mutation directly by preventing accurate replication of the DNA at that site. Alternatively, faulty repair of the altered DNA by cellular enzymes may lead to a modification of the DNA that is perpuated in all subsequent generation.The mutation may cause a readily observable alteration in cellular structure or function, or both (Miller and Miller, 1981).

Carcinogenesis is a multistep process and requires the passage of time. The great majority of chemical carcinogenesis and their metabolites are strong electrophillic reactants and these reactants can react with cellular DNA to cause mutation (Miller and Miller, 1981; Singer and Kusmierek, 1982). Chemical mutagens are also the agents that capable

of causing a change in genetic material that can be inherited. Some mutagens are electrophiles thus can interact directly with DNA, others have to be metabolized by enzymatic reaction into electrophillic intermediates, then form covalent bonds with cellular nucleophiles. Carcinogens and mutagens may be classified as genotoxic agents. Chemical carcinogens, or indeed, chemical mutagens, exert their adverse biological effects by involving heritable alteration in genetic structure (Wright, 1980). Most of the classical carcinogens were mutagens that caused damage to DNA (McCann et al., 1975a; McCann and Ames, 1976). There is an exisiting circumstantial evidence that mutagenesis may be an initial stage in carcinogenesis. (Howard-Flanders, 1981, and Mohn, 1981). Experimental evidence supports the theory that mutagenicity is indeed part of the carcinogenic process (Ames, 1989a).

1.4 Mechanism of Inhibition of mutagenesis.

Several known or suspected carcinogenic agent may be mutagenic changes in DNA, it means that the inhibition of carcinogen-induced mutation is a good indicator of anticarcinogenic properties. The term "antimutagenic" was used to describe agent that reduce the apparent yield of spontaneous and/or induced mutation, regardless of the

mechanism involved. Because mutagenesis is not only one step but it is a multistep and used more than one way to complete, the "antimutagens" is not only one type either, it includes "desmutagens" and "bioantimutagens" (Kada, et al., 1982). So antimutagenesis can be divided into two different processess, desmutagenesis and bioantimutagenesis.

" Desmutagens" are antimutagenic agents acting outside the cell by acting directly on mutagens or their precursors and inactiving them. The mechanism of desmutagenesis involves; chemical inactivation of mutagens, enzymatic inactivation of mutagens, inhibition of metabolic activation of promutagens and inactivation of activated mutagens.

" Bio-antimutagens" are antimutagenic agents acting inside the cell by acting on the process of mutagenesis or repair DNA damage to result in decreasing mutation. The mechanism of bioantimutagenesis involves; mutation induction and repair process. The mechanism of desmutagenesis and bioantimutagenesis is shown in Figure 1.

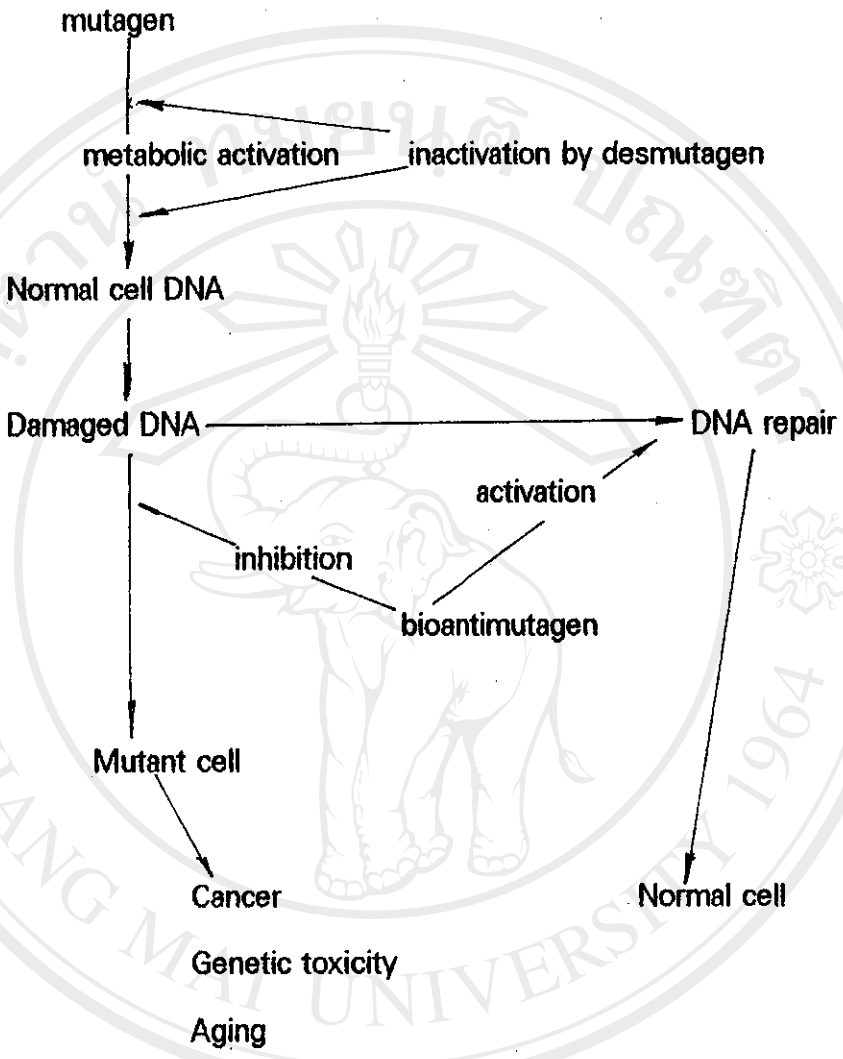

Figure 1. Schematic showing the process of antimutagenesis.
(derived from Kada, and Shimoi, 1987)

1.5 Dietary inhibitors of mutagenesis and carcinogenesis

Dietary inhibitor of mutagenesis and carcinogenesis are important because they may be useful for human cancer prevention. The search for mutagenesis inhibitor is to for discovering antimutagenic agentic (Hayatsu, et al., 1988 ; and Ramel, et al., 1986). Many mutagenesis inhibitors have been discovered by the use of short-term assay as the Ames test (Jacobs, et al., 1977, Rosin and Stich, 1978 ; Rosin and Stich, 1978 ; and Kada, et al., 1978). The discovery of cancer chemopreventive agents is an important area for cancer control and prevention (Hinnekeks, 1980., Higginson, 1983, and Higginson, 1988).

There are various chemical substances in diet that have been reported to be inhibitors of mutagenesis and carcinogenesis. the substances that are considered to be constituents of natural foods : those in meats, egg, cereals, fats, oils, vegetables, fruits, beverages (tea, coffee, milk, juice, alcohol, etc., and others in diet. Dietary inhibitors include porphyllins, fatty acids, vitamins, polyphenols, sulfhydryl compound, Vitamin A, E and C, ellagic acid, fats, selenium, calcium and fiber.

Porphyllins from plant, both chlorophyll and chlorophyllin, can inhibit activities of some mutagens. Oleic acid was shown to inhibit the mutagenic activity of food pyrolysate

mutagens (Trp-P-1, Trp-P-2 and others), polycyclic aromatic hydrocarbons and nitrosamines. Some compounds that occur in plants ellagic acid, was showed antimutagenic activity against B (a)p-diol epoxicde . Sulfhydryl compoun, glutathione and cysteamine are inhibitors of the direct-acting mutagen 2-acetoxyacetylaminofluorene. Cystein inhibits the mutagenicity of 2-acetoxyacotylaminofluorene and that of N -hydroxyacetylamino-fluorene. (Hayatsu, et al., 1988).

Several mutagenesis inhibitor have been demonstrated to be carcinogenesis inhibitors as well, The examples are ellagic acid, palmitoleic acid, and N-acetylcysteine. This indicates that the search for mutagenesis inhibitors will be useful for further discovering anticarcinogenic agents.

1.6 Bacterial mutation assay.

Many biological assays have been used to detect chemicals that potentially produce cancer and genetic damage (mutations). It was found that about 85% of the carcinogens tested have been detected as mutagens. (Ames, et al., 1973; Ames, et al., 1975; Ashby, 1989; Liginsky, 1989; McCann and Ames, 1976; Miller and Miller 1981, and Ramel, 1986). Bacterial mutation assay, or the Salmonella /microsome mutagenicity test (Ames, et al.,1975), is a short-term test which is supersensitive and simple . Bacterial test systems can be divided into 3 main classes, those are , (Maron and Ames, 1983)

1. those that detect forward mutations,
2. those that detect backward mutations,
3. those that rely on a DNA repair deficiency.

Bacterial mutation assay using backward (or reverse) mutation is the most widely used of all methods for determining the mutagenicity of chemicals. The bacterial strains (Salmonella typimurium) used in reverse mutation tests cannot synthesize amino acid histidine from inorganic source of nitrogen and are therefore designated as His^-. This assay determines whether the test chemical can reverse the effect of the pre-

-existing mutation (His^-) by causing a second mutation which allows the bacterium to synthesize histidine from inorganic nitrogen. The resulting mutants are also called revertants, because the bacterial strains used in this assay lack the enzymes necessary for metabolising pro-carcinogens to ultimate carcinogens. Rat liver S9 is added as crude surrogate of mammalian metabolism.

The short-term tests "Bacteria mutation assay" have been used not only to identify mutagens and potential carcinogens but are increasingly being used to identify antimutagens and potential anticarcinogens (Sugimura, 1988)

1.7Statement of Problem

Many thousands of chemicals are present in the environment are known to be toxic. The toxic characteristics of a chemical may be described in term of its quantitative types of toxicity, e.g., neurotoxins, hepatotoxins, carcinogens, and mutagens, etc. and in term of carcinogens and mutagens, toxic agents which are in daily life are industrial products, medicines, domestic products, cosmetics, pesticides, insecticides, and foods, etc (IARC, suppl., 1987)

Many studies showed that diets and life-styles are closely related to mutagenesis, carcinogenesis and human cancer(Doll and Peto, 1981). Diet is a complex mixture that not contain only mutagens, or carcinogens but also contains antimutagens, anticarcinogens and factors that can affect the process of mutagenesis and carcinogenesis (Ames, 1989a). Therefore,understanding of the mechanism of the inhibition is necessary for cancer prevention. From many reports, about carcinogenic agents it was found that about 85% of the carcinogens tested have been detected as mutagens (McCann and Ames, 1976).

Bacterial short-term assay have been used to identify mutagens, potential carcinogens, as well as antimutagens and anticarcinogens.

1.8 Purpose of the study.

The investigator was interested in environmental dietary factors which may be able to prevent or protect cancer formation. The investigation will be focussed on the presence of natural antimutagens and/or anticarcinogens in Thai foods.

Foodstuffs commonly consumed by Thais have been show totoи to contain some mutagenic compounds to Salmonella typhimurium strain TA 98 and TA 100 (Purintrapibal, 1989; and Vinitketkumnuen,1984). Interestingly, the results have suggested that lemon grass (Cymbopogon citratus Stapf) contained some antimutagenic activity. Therefore, it is considered worthy to investigate the active antimutagenic agents in lemon grass.

Lemon grass (Cymbopogon citratus Stapf) is commonly used as ingredient for cooking inThailand. It is an aromatic grey-green grass which belong to the family Graminae . The bases of the stem are used in cookeries. It is commonly used as a Thai medicinal plant too.

Foods contains many mutagenic substances which may be potential carcinogens but foods also contain protective substances which are belived to combat against cancer (Ames, 1983; Lai, et al, 1979). The antimutagenic activities of Thai vegetables were previously reported (Rojanapo, and Tepsuwan., 1992) The identification of the antimutagenic substances in Thai foods is still of immense importance. There is obvious interest in determining whether current dietary recommendations are sufficient to provide the necessary anticarcinogenic capacity for optimal protection against carcinogenesis. We have to know more about micronutrients in foods that have antimutagenic/and or anticarcinogenic property. When the assay of antimutageniccity information is done the complex relationship between dietary intake and the possible protection would be correctty interpreted. The purposes of this study were

1. To determine antimutagenic activity of lemon grass extract in Salmonella mutation assay.
2. To study the possible mechanism of inhibition of lemon grass extract against AFB_1, and MNNG- induced mutation in Salmonella.
3. To partially purify active antimutagenic substance from lemon grass.