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thesis entitled

Determination of the Prostaglandin E Metabolite (PGE-M)
By Gas Chromatography-Mass Spectrometry and Excretion
of PGE-M By Women Consuming a Garlic Extract

presented by

Ching-Hsun Chiou

has been accepted towards fulfillment
of the requirements for

Masters degree in Science

Maurice R. Bennink
Major professor

Date 2-23-95

O-7639

MSU is an Affirmative Action/Equal Opportunity Institution

DETERMINATION OF THE PROSTAGLANDIN E METABOLITE (PGE-M) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND EXCRETION OF PGE-M BY WOMEN CONSUMING A GARLIC EXTRACT

BY

CHING-HSUN CHIOU

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1995

ABSTRACT

DETERMINATION OF THE PROSTAGLANDIN E METABOLITE (PGE-M) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND EXCRETION OF PGE-M BY WOMEN CONSUMING A GARLIC EXTRACT

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A study was conducted to develop a method to quantitate urinary excretion of PGE-M, an indicator of whole body turnover of prostaglandin E_1 and E_2. PGE-M excretion in one individual taking aspirin and eight women consuming a garlic extract (GE) were also examined. PGE-M and $[^2H_7]$-PGE-M (internal standard) were co-extracted from 20 ml of urine, methylated, partially purified, methoximated, and trimethylsilated. PGE-M was analyzed by gas chromatography-mass spectrometry using the selected ion monitoring mode at m/z 365 and m/z 372. Aspirin but not GE consumption decreased PGE-M excretion. There was no difference in PGE-M excretion before (42.7 ± 9.5 μg/24 hr) and after three months of garlic consumption (35.9 ± 4.7 μg/24 hr) ($P = 0.29$). It is concluded that the GE did not contain sufficient prostaglandin inhibitors to reduce PGE-M excretion.

ACKNOWLEDGMENTS

I would like to sincerely thank my major professor, Dr. Maurice R. Bennink, for his support and guidance throughout this study. Appreciation is also expressed to Dr. Dale R. Romsos and Dr. Wanda E. Chenoweth for serving on my guidance committee.

Thanks to Dr. J. T. Watson and everyone who have assisted me in the mass-spectrometry facility room in the Biochemistry department.

I also appreciate my parents, family and friends who always stand by me and give me their support and encouragement.

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INTRODUCTION

Cancer has become a major cause of death all over the world during the last several decades. The incidence of several kinds of cancer, such as colon, breast, ovary, prostate and endometrial, has been related to diet as shown by both epidemiological and laboratory studies (Weisburger and Rivenson, 1993). For example, colon cancer, the third most common malignant cancer in the United States, was found to be associated with high intakes of fat and animal protein, and with low intakes of fiber. People who consume high fat diets also have higher incidence of breast, pancreatic and colorectal cancers.

Carcinogens can be formed during food preparation. Examples are polycyclic aromatic hydrocarbons and heterocylic aromatic amines - compounds that can initiate mammary and colon cancer in experimental animals. Conversely, many possible cancer preventive components have been found in the diet, particularly in foods from plant sources. Foods with chemopreventive potential include garlic, soybean, licorice, green tea, umbelliferous vegetables, and citrus fruits (Caragay, 1992).

In fact, a variety of anti-carcinogens and potential carcinogens co-exist in our diet, and these naturally occurring substances in foods can affect cancer initiation, promotion, and progression by different mechanisms. The dietary anti-carcinogens suppress carcinogenesis development by inducing biotransforming enzymes that block initiation and reduce carcinogenicity, or by inhibiting tumor promotion (Davis, 1989).

Oxidative damage, prostaglandins and related eicosanoids play an important role in the tumor promotion stage of carcinogenesis. The promotion process can be blocked by compounds such as phenolics, salicylates, flavonoids, polyacetylenes, and sulfides. Retinols and tocopherols found in vegetables and animal foods, and organosulfates found in garlic and onion, also inhibit tumor promotion (Hayes et al., 1987; Wargovich et al., 1987; Sparnins et al., 1988).

The Experimental Foods Program from the National Cancer Institute (NCI) identified over forty foods with cancer preventive activity, and garlic was considered the food with the most anti-cancer potential. The ancient pharmacological and health promoting attributes of garlic have been known for thousands of years. In addition, modern science suggests that several of the organosulfur compounds in garlic are biologically active and elicit a number of physiological functions, including inhibition of prostaglandin biosynthesis and inhibition of cancer (Davis, 1989; Lau et al., 1993).

The objective of this study was to develop a method to determine the prostaglandin E metabolite in urine and to determine if a garlic extract would suppress prostaglandin E production in humans. The quantitative measurement of prostaglandin E metabolite (PGE-M) excretion in urine, which is an indicator of whole body biosynthesis of prostaglandin E_1 and E_2, was used to assess the effect of the garlic extract on prostaglandin biosynthesis.

REVIEW OF LITERATURE

A. BIOLOGY OF EICOSANOIDS

A.1. Precursors of Eicosanoids

Prostaglandins, leukotrienes, and related hydroxy fatty acids are members of the eicosanoid family that accounts for a variety of significant physiological functions in humans. Dihomo-gamma-linolenate (20:5ω6), arachidonic acid (AA, 20:4ω6), and eicosapentaenolate (20:5ω3) are immediate precursors for eicosanoid biosynthesis. Of these, AA is the main unsaturated fatty acid substrate used for eicosanoid synthesis in most mammalian cells.

AA is obtained either directly from the diet, mainly from animal products, or AA is synthesized from linoleic acid, via desaturation and elongation reactions. Once AA is synthesized, it is incorporated into cell membrane phospholipids. AA is esterified primarily to the β-position of phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine or phosphotidylserine.

Since only unesterified polyunsaturated precursors can enter the eicosanoid formation cascade (Struyck et al.,1966), the release of non-esterified arachidonic acid (NEAA) is an important point for regulation of eicosanoid biosynthesis

(Smith, 1989). AA release from membranes is relatively quick and is typically accompanied by turnover of inositol containing lipids (Lapetina et al., 1978). Deesterification by phospholipase A_2 (PLA_2), phosphololipase C (PLC) and diacylglyceral lipase occurs in response to particular hormone stimuli in specific cells.

Phospholipase A_2 is the key enzyme which catalyzes the breakage of the β-bond to release AA from phospholipids; thus stimulation of PLA_2 plays a pivotal role in AA release. The mechanism of AA release is not the same as for the release of fatty acids from triglycerides in adipose tissue. Fatty acids release from adipose tissue is regulated by hormone sensitive lipase (Hagenfeldt et al., 1975a). The release of AA from phospholipids is increased by stress (Deby-Dupont et al.,1983) but is not affected by exercise (Hagenfeldt and Wahzen, 1975b). Plasma non-esterified fatty acids other than AA and from adipose tissue are increased by both stress and exercise.

A.2. Regulation of the Eicosanoid Precursors Release

The biochemical details involved in the deesterification process have not been well resolved. Many types of hormones, autocoids, growth factors and tumor promoters elicit AA release by receptor mediated action. Alternately, mechanical stress on the cell such as shear forces acting on vascular endothelial cells can produce NEAA. Hormones such as angiotensin II (Nolan et al., 1981), bradykinin (Hong and Levine, 1976) and catecholamines (Norcia and Evans, 1962) are stimulators of PLA_2. Glucocorticoids (Di Rosa and Persico, 1979; Flower and Blackwell, 1979), and lipomodulin protein (Hirata, 1982) inhibit PLA_2 activity. Cellular calcium and cyclic AMP (cAMP) regulate AA release also. For example, cAMP can inhibit the release of eicosanoid precursors from membrane phospholipids by decreasing phospholipase activity (Minkes et al., 1977). Ca^{2+} stimulates phospholipase activity and inhibits adenylcyclase while cAMP decreases cytosolic Ca^{2+} concentration (Gemsa et al., 1979; Lim et al., 1981).

A.3. Biosynthesis of Eicosanoids

Once deesterified from phospholipids, free AA can be oxidized and further metabolized to a variety of biologically active C_{20} eicosanoid products (Figure 1). The formation of eicosanoids is species specific and tissue specific as well (Sun et al., 1977). In mammalian cells, almost all cells except red blood cells (RBC) can produce prostaglandins and related compounds in response to a particular stimuli (Voet, 1990). Depending on the specific cells, there are three different pathways for AA oxygenation- the cyclic pathway leads to the formation of prostaglandins and thromboxanes (prostanoids), the lipoxygenase pathway leads to the synthesis of leukotrienes, mono-, di-, and tri- hydroxy acids, and the epoxygenase pathway to the biosynthesis of epoxides. Details for the biosynthesis of prostanoids will be discussed in more detail in section B.1.

Eicosanoids are formed in response to various stimuli to

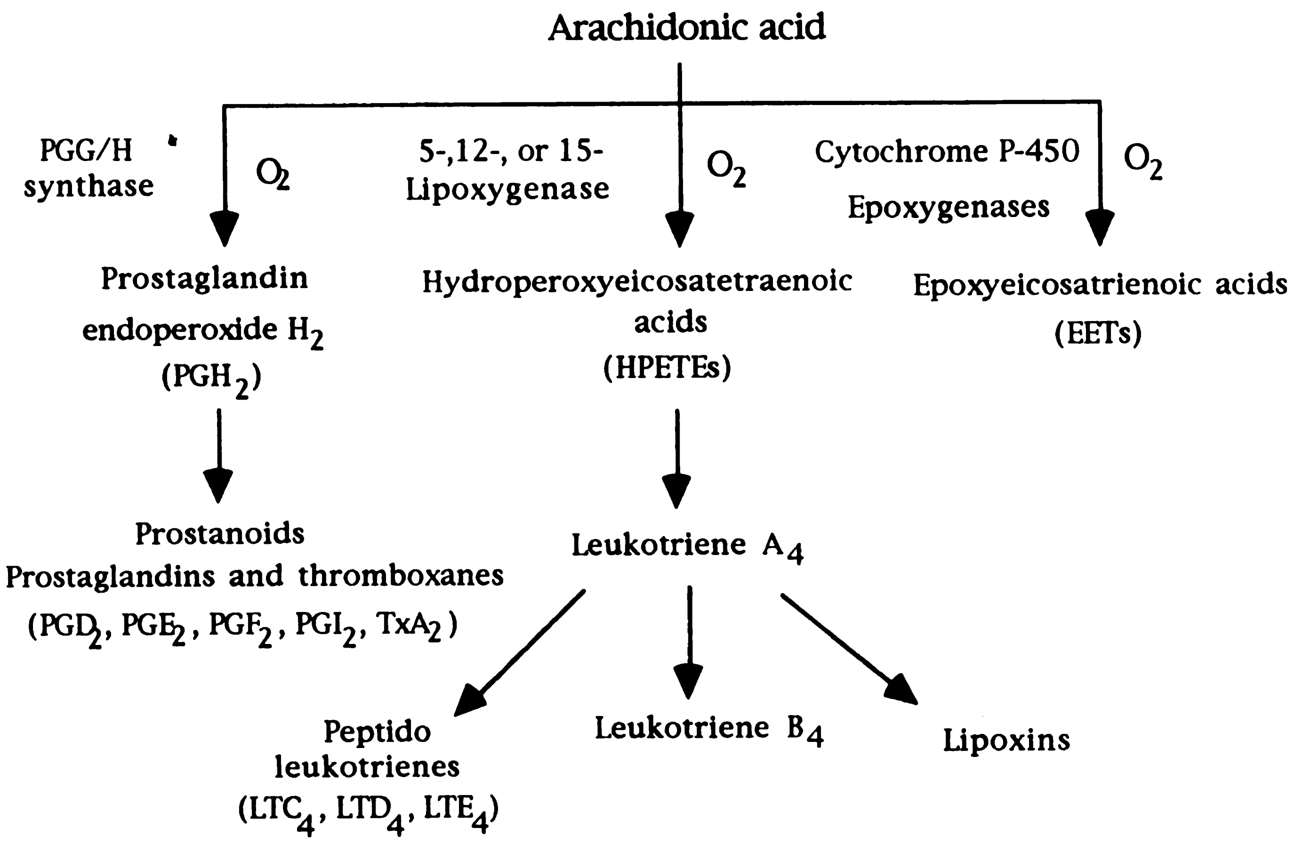


Figure 1. Arachidonate cascade.

(Adapted from Smith, 1989)

generate a variety of general biological functions or specific physiological responses resembling steroid hormone activity. Similar to hormones, extremely low concentrations of eicosanoids are sufficient to elicit physiological effects. However, unlike hormones, the eicosanoids are not transported through the blood stream to target sites; rather, they are unstable substances utilized rapidly inside the cell where they are produced. In other words, the eicosanoids act as local hormones that are synthesized in the cell and function within the same environment (Ferreia and Vane, 1967).

A.4. Physiological Function of Eicosanoids

Since eicosanoids are synthesized in most tissues, their biological activities are broadly distributed in most organs, i.e., cardiovascular, renal, reproductive, respiratory, digestive, and neurotropic systems. Besides, eicosanoids are also involved in platelet aggregation, pain and fever production, inflammatory response, induction of blood clotting, reproduction control, and sleep/wake cycle regulation, etc. (Voet, 1990). The targets and functional activities of eicosanoids may be associated with their structural characteristics, i.e., their hydrophilic or lipophilic properties (Euler, 1963). In some cases, different eicosanoid families are antagonist to each other. For example, in platelets, thromboxane A_2 (TxA_2) acts as a vasoconstrictor and stimulator of platelet aggregation, whereas prostaglandin I_2 (PGI_2) is a vasodilator and an inhibitor of platelet aggregation. In normal conditions, homeostatic mechanism maintain prostanoid balance in the cardiovascular system.

B. THE E SERIES OF PROSTAGLANDINS (PGE)

B.1. Biosynthesis of PGE

Among eicosanoids, prostaglandin E_2 (PGE_2) is characterized as the most common and most potent mammalian prostaglandin synthesized in the cyclic pathway of arachidonic acid metabolism (Figure 2). The first step of arachidonic acid oxygenation is mediated by prostaglandin synthetase which has two enzyme activities - cyclooxygenase activity involved in PGG_2 formation by adding two molecules of oxygen to AA and hydroperoxidase activity which adds two electrons to reduce PGG_2 to yield PGH_2.

The cyclooxygenase enzyme activity is specifically inhibited by non-steroid anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, ibuprofen, phenylbutazone, etc. (Vane et al., 1971; Flower, 1974; Gryglewski, 1975). Many NSAIDs are therapeutically effective in relieving pain, fever and general inflammatory response by inhibition of prostanoid biosynthesis. NSAIDs are commonly utilized as prostanoid biosynthesis inhibitors in laboratory studies.

Since cyclooxygenase is ubiquitous in human nucleated cells, prostanoids (prostaglandins and thromboxanes) are generally produced in most cells with the exception of red blood cells. As mentioned earlier, prostaglandins act like

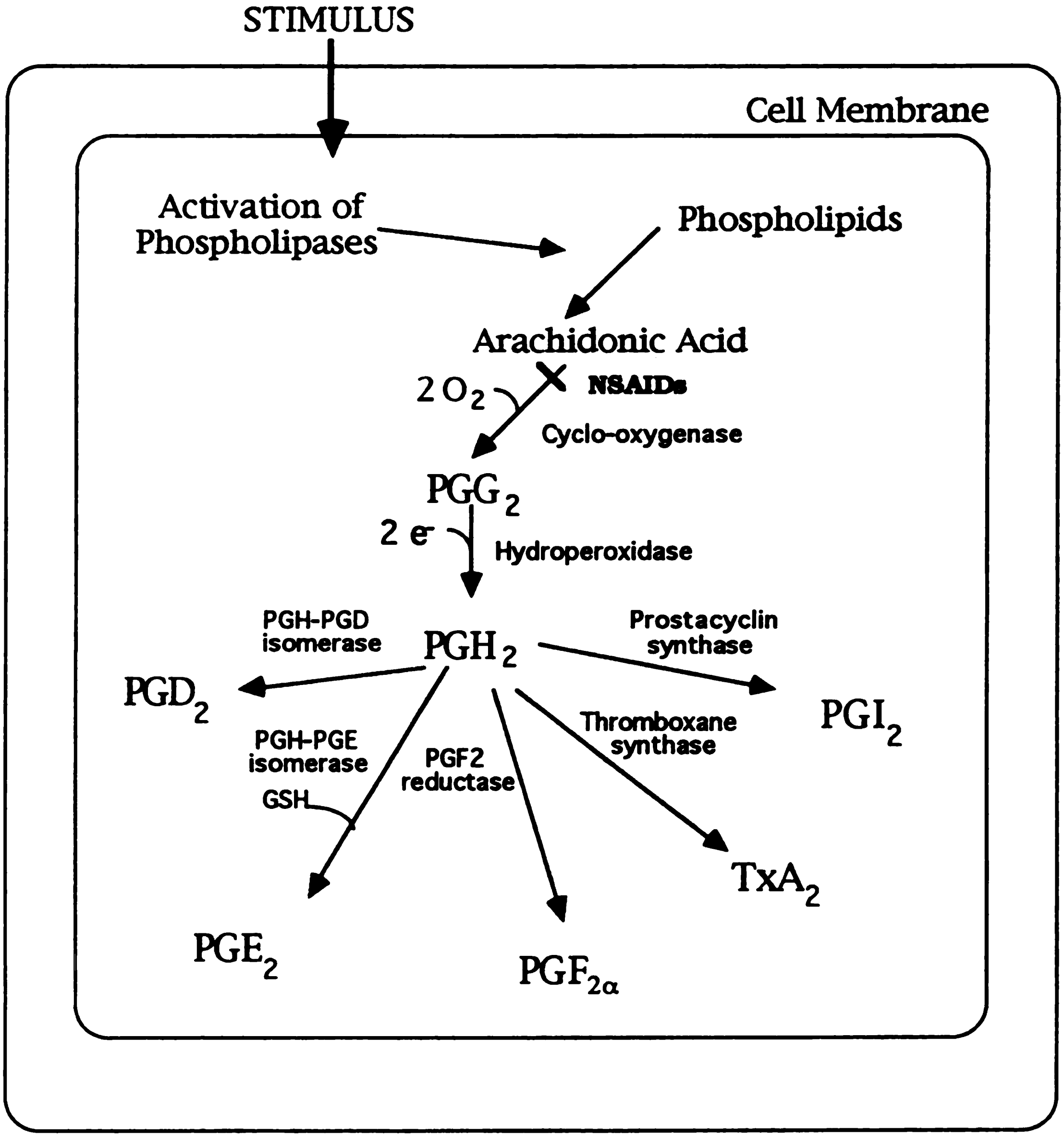


Figure 2. Biosynthetic pathway for prostanoid formation. The cyclooxygenase activity is inhibited by non-steroid anti-inflammatory drugs (NSAIDs).

(Adapted from Smith, 1989)

local hormones. Thus, formation of specific prostaglandins depends upon tissue need and presence of enzymes for specific prostanoid synthesis.

Focusing on PGE_2, prostaglandin endoperoxidase E isomerase (PGH_2/PGE_2 isomerase) is responsible for converting PGH_2 to PGE_2. This enzyme was extensively purified from sheep seminal vesicle microsomes and found to be a glutathione dependent enzyme (Ogino et al., 1977).

B.2. Catabolism of PG

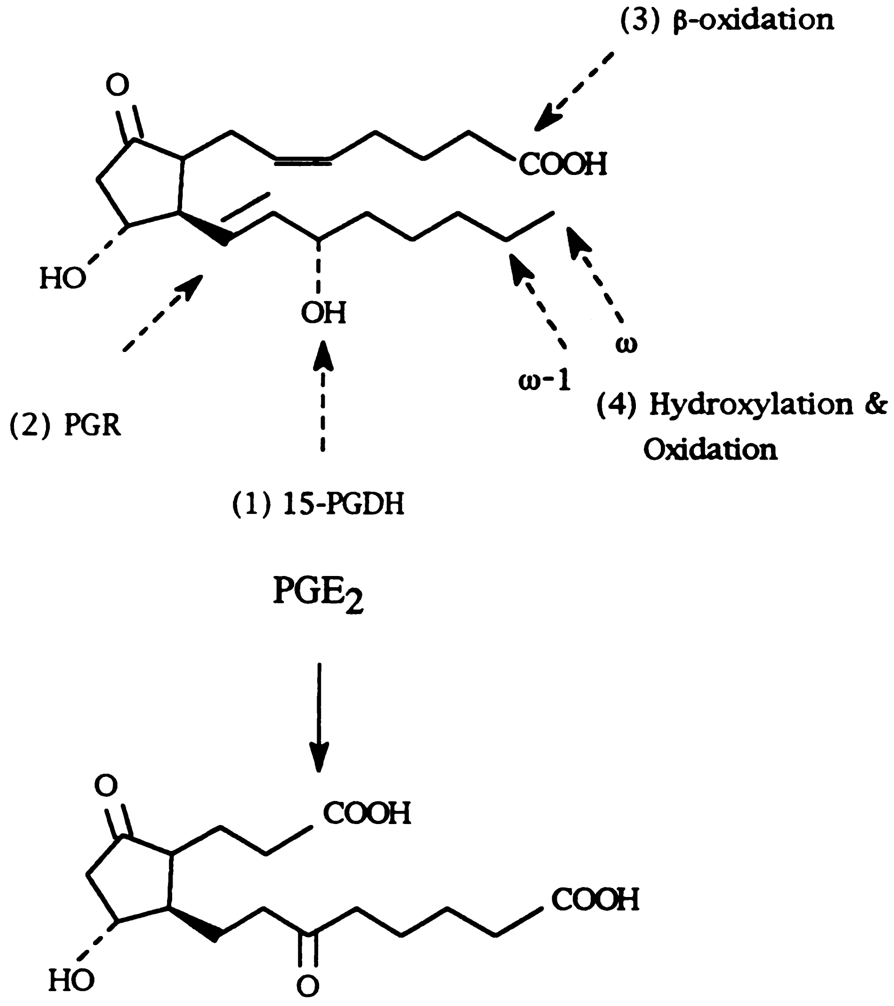
Normally, prostanoids are efficiently catabolized in tissues where they are formed. However, the lung play an important role in PG uptake and catabolism as well. The lungs serve to "filter" biologically active substances from the blood stream to ensure that these compounds are at optimum concentrations in the arterial circulation. If excess prostaglandins are synthesized in peripheral tissues, the lung inactives and degradates prostanoids. This process is very efficient since 80-95% of PGE_2 and $PGF_{2\alpha}$ is catabolized in lung of guinea pig, rat, rabbit, cat, dog, and human (Piper et al., 1970).

The initial step in PG catabolism by lung is PG uptake from extracellular space into the cytoplasm where PG degrading enzymes are located (Bito et al., 1977). PGs are transported across the plasma membrane by a carrier mechanism since PGs do not freely pass across biological membranes. All PGs compete for a single carrier mechanism (Eling and Ally, 1981).

The enzyme responsible for classical prostaglandin catabolism is 15-hydroxy prostaglandin dehydrogenase (15-PGDH) (step 1 in Figure 3). Two types of 15-PGDH have been identified (Nakano, 1969). Type I (15-PGDH), NAD^+ dependent enzyme, is present in most tissues including lung, spleen, parts of gastro-intestinal tract, kidney and heart. Type II (15-PGDH) enzyme, which needs $NADP^+$ as a cofactor is found in kidney, brain, and erythrocytes. Among PGs, PGE_2 is the best substrate for both types of 15-PGDH.

After the PGs are oxidized to 15-keto PGs by 15-PGDH, the next enzyme involved in catabolism is prostaglandin reductase (PGR; step 2 in Figure 3). PGR is an NADH or NADPH dependent enzyme, which reduces the C13-C14 double bond to yield 13, 14-dihydro-15-oxo-prostaglandin derivatives, the major metabolites found in plasma and lung effluents. The 13, 14-dihydro-15-oxo-PGE_2 has very little biological activity and a 8 min half life in blood circulation in man (Hamberg and Samuelsson, 1971). In comparison, PGE_2 has a 30 second half life. Further metabolism is mediated by ß or ω-oxidizing enzymes (step 3 and 4, Figure 3) in liver, kidney, and intestine (Sammuelson et al., 1971). These enzymes are not specific for prostaglandins, they act on many fatty acids. The final metabolite, 11α-hydroxy-9,15,-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid (PGE-M), is excreted in the urine (Hamberg and Samuelsson, 1971; Ferretti et al., 1987).

The level of urinary PGE-M is a better indicator of



PGE-M

11α-hydroxy-9,15,-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid

Figure 3. Sequence of reactions involved in the degradation of PGE_2 and formation of the major urinary metabolite (PGE-M). 15-PDGH: 15-hydroxy-dehydrogenase, PGR: Prostaglandin reductase.

whole body PGE turnover than PGE concentrations in plasma or urine. Considering that white blood cells, platelets, kidney, and prostate cells produce PGE, measuring PGE in blood or urine does not accurately reflect whole body turnover for prostaglandins. Assessment of urinary PGE-M could be a valuable indicator for nutrition and clinical modulation studies that are designed to modulate PGE turnover (Ferretti et al., 1988; Ferretti et al., 1989; Schweer et al, 1990).

B.3. Quantitative analysis of PGE-M by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is a powerful microanalytical technique that combines two sample analyzers - a gas chromatograph that separates analytes, and a mass spectrometer that causes the analytes to decompose into fragments which can then be used for sample identification (Watson, 1985). Various strategies are available to optimize analyte identification and quantification. For example, in the total ion current (TIC) chromatography mode, the mass analyzer records the abundance of all ions generated by analyte degradation. TIC provides an overall ion performance of the sample. The selected ion monitoring (SIM) mode records only specific mass-to-charge (m/z) values that have been selected because they characterize the analyte of choice. The SIM technique features high selectivity and high sensitivity and is ideal for quantitative assays of biological samples (Seulter and Watson, 1990).

B.4. Biological Function of PGE

Prostaglandin E_1 (PGE_1) and Prostaglandin E_2 (PGE_2) are the major PGs of the E series and they are found in relatively high concentrations in the renal system, coronary tissues, and immune cells. Thus, the physiological activities of PGE are closely associated with renal function, blood pressure regulation, and immune response modulation. Not only PGE_2, but all the prostaglandins and leukotrienes, conduct their physiological operation through the G protein-linked receptors. The role of PGE_2 in water reabsorption in renal collecting tubules represents a typical model of eicosanoid action (Smith, 1989). Immuno-regulation is an important normal function of PGEs also. However, excessive prostaglandin production is considered to promote carcinogenesis.

B.5. PGE and Tumorgenesis

More and more studies have shown that PGs may play a role in tumorgenesis, especially in tumor promotion. However, discerning the role of PGE in tumor promotion is very complicated since PGE is widely distributed throughout the body and often PG synthesis is under tissue specific regulation. Besides, low or high doses of PGs may exhibit different or even opposite effects on tumor regulation; both tumor inhibition and tumor promotion effects have been suggested. For example, in many tumor cell lines, PG synthesis is persistently raised, particularly the E series PGs (Karmali et al., 1979; Bennett et al., 1979). *In vivio* it is not known if the increase of PGs are produced by tumor cells or by host-derived macrophages (Evans, 1973; Svennevig and Svarr, 1979).

Phorbol esters, such as TPA, are potent tumor promoters. TPA leads to the activation of PLA_2 and enhances PG synthesis; the tumor promoting effect of TPA may be in part due to increased PG synthesis.

C. REGULATION OF PROSTAGLANDIN METABOLISM

C.1. Stimulators of Prostaglandin Synthesis

Under normal circumstances, the prostaglandins are maintained at specific levels for their tissue specific physiological functions; however, in many occasions, cells may over-produce PGs and related eicosanoids and cause improper physiological performance. Stimulators of PG synthesis include hypersensitivity mediators such as cytokines and lymphokines (Rankin et al., 1982), platelet activating factor (Voelkel et al., 1982), ultraviolet light (Morris et al., 1978), calcium ionophores (Jakschik et al., 1977), etc. In addition, as mentioned earlier, PG synthesis in many tumor cell lines is significantly increased; particularly the E series of PGs (Karmali et al., 1979; Bennett et al., 1979). Thus, compounds that can affect eicosanoid metabolism are important modulating reagents when the body over-produces these physiologically active eicosanoids.

C.2. Inhibitors of prostaglandin synthesis

Drugs used to inhibit prostaglandin synthesis affect different targets in the eicosanoid biosynthetic pathway. Steroid hormones act up stream of AA metabolism by inducing synthesis of proteins that inhibit PLA_2. Consequently, steroid hormones reduce the availability of PG precursors by inhibiting AA release from phospholipids (Flower and Blackwell, 1979). In addition, cyclooxygenase activity can be inhibited by various drugs. These include the nonsteroid anti-inflammatory drugs (NSAIDs) (Vane, 1971), the eicosa-5,8,11,14 tetraenoic acids which act as analogs of AA (Ahern and Douing, 1970), antioxidants (Gwebo, 1978; Bauman et al., 1980), some phenolic compounds (Baumann et al., 1979), and compounds in garlic and onion (Vanderhoek et al., 1980). These substances can down regulate excessive production of arachidonic acid metabolites and thereby modulate the physiological abnormalities due to over-production of prostanoids.

From a nutrition point of view, it is valuable to identify dietary compounds that promote health and that may have pharmacological activity. Garlic possess these advantages and has been utilized for these purposes for thousands of years. The Experimental Foods Program of the National Cancer Institute (NCI) points out that over forty foods with cancer preventive potential have been identified. These food include garlic, cabbage, licorice, soybeans, ginger, and umbelliferae; garlic is considered to have the greatest anticancer potential (Caragay, 1992) (Figure 4). The anti-cancer properties of garlic is associated with inhibition of prostaglandin synthesis (Srivastava and Tyagi, 1993; Ali et al., 1993). Additional supportive studies will be discussed in section D.

D. PHARMACOLOGICAL EFFECT OF GARLIC

D.1. Introduction

For thousands of years, garlic (*Allium sativum*) has been used as a herbal medicine for a variety of ailments including headache, heart problems, animal bites, intestinal parasites, etc. (Block, 1985). Recent studies demonstrate that compounds in garlic produce remarkable biological actions that may correlate with the pharmacological effects noted in folklore.

D.2. The Chemistry of Garlic

In 1944, Cavallito et al. (1944) produced an ethyl alcohol extract of garlic that had anti-bacteria and anti-fungal properties (Cavallito et al., 1944; Appleton and Tansey, 1975; Barone and Tansey, 1977). Later studies have shown that dietary garlic oil reduced the severity of atherosclerosis in rabbits (Bordia et al., 1977) and serum cholesterol in rat (Sodimu, 1984). Several researchers reported that various garlic extracts inhibit human platelet aggregation by a variety of mechanisms (Bordia, 1978; Vanderhoek et al., 1980; Aptiz-Castro et al., 1983; Mohammad, 1986; Mayeux et al., 1988; Shalinsky et al., 1989).

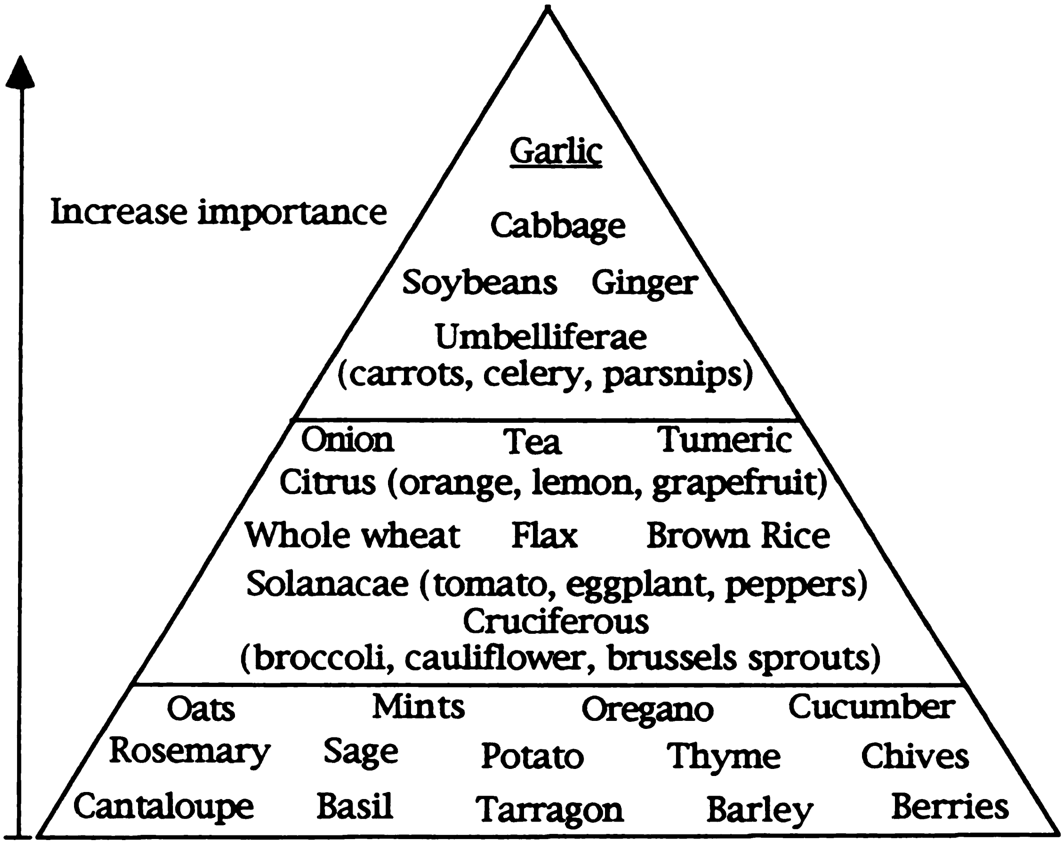


Figure 4. Possible cancer-preventive foods and ingredients.

(Adapted from Caragay, 1992)

Many kinds of organosulfur compounds have been identified in garlic; some of these compounds account for the potent biological activities attributed to garlic (Block, 1985; Dorant, 1993) (Table 1). In fresh garlic, the majority of the organosulfur compounds exist as γ-Glutamyl-S-allylcysteine (GSAC) (Figure 5). GSAC can be proteolytically hydrolyzed to produce alliin during storage of the garlic cloves. When a clove is crushed or cut, the enzyme allinase is activated and it converts alliin to allicin, the source of garlic odor. Allicin is responsible for much of the anti-bacterial and anti-fungal properties of garlic. Allicin is not stable and it self-condenses to form another potent compound, ajoene. Ajoene was found to be an effective anti-thrombotic agent that prevents platelet aggregation (Block et al., 1986). Other compounds such as diallylsulfides, allixin and even crude garlic extracts inhibit aflatoxin B1 induced mutagenesis (Yamasaki et al., 1991).

D.3. Garlic inhibit Arachidonic Acid Metabolism

The anti-thrombotic effect of garlic was associated with arachidonate metabolism. It has been found that the 5-lipoxygenase and prostaglandin synthetase activity were strongly inhibited by ajoene (Wagner et al., 1987). An anti-platelet aggregation event was triggered by ajoene possibily by the alteration of AA metabolism (Srivastava and Tyagi, 1983). Chemically synthesized allicin also showed an inhibitory effect on platelet aggregation and neutrophil enzyme release, but no direct effect on cyclooxygenase,

Table 1. Organosulfur compounds in garlic tested in vitro and in vivo assays for possible anti-carcinogenic activity.

Chemical structure	Compound	Abbr.
$CH_2=CH-CH_2-S(O)-CH_2-CH(NH_2)-COOH$	Alliin	
$CH_2=CH-CH_2-S(O)-S-CH_2-CH=CH_2$	Allicin	
$CH_2=CH-CH_2-S(O)-CH_2-CH=CH-S-S-CH_2-CH=CH_2$	Ajoene	
$CH_2=CH-CH_2-S-CH_2-CH=CH_2$	Diallylsulfide	DAS
$CH_2=CH-CH_2-S-S-CH_2-CH=CH_2$	Diallyldisulfide	DADS
$CH_2=CH-CH_2-S-S-S-CH_2-CH=CH_2$	Diallyltrisulfide	DAT
$CH_2=CH-CH_2-S-S-CH_3$	Allylmethyldisulfide	AMD
$CH_2=CH-CH_2-S-S-S-CH_3$	Allylmethyltrisulfide	AMT
$CH_2=CH-CH_2-SH$	Allylmercaptan	AM
$CH_2=CH-CH_2-S-CH_2-CH(NH_2)-COOH$	S-allylcysteine	SAC

(Adapted from Dorant, 1991)

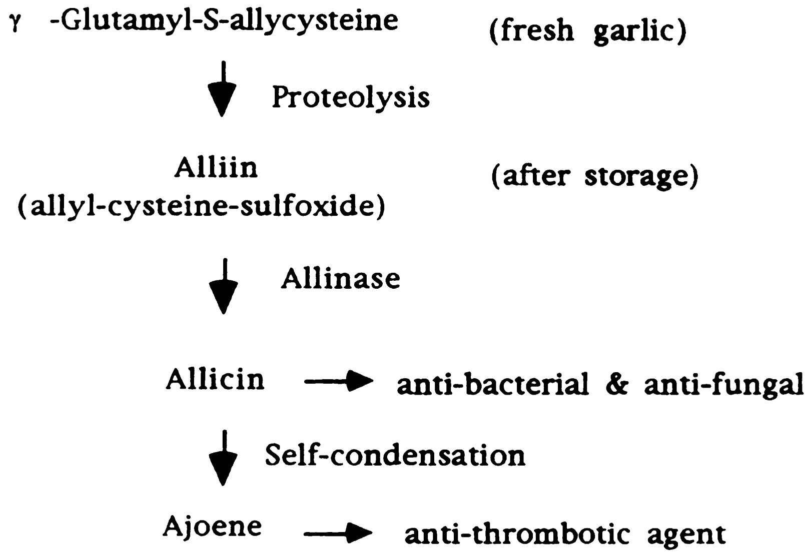


Figure 5. Organosulfur compound transformation in garlic.

thromboxane synthase, or prostacyclin synthase from a number of different cell sources (Mayeux et al., 1988). A later study confirmed that allicin did not alter cyclooxygenase activity, but showed a dose dependent reduction of PGE_2 formation, suggesting that allicin may act on the glutathione dependent PGH_2/PGE_2 isomerase, the enzyme responsible for PGE_2 biosynthesis (Shalinsky et al., 1989). The prostaglandin synthesis in the ovine ureter was also inhibited by an aqueous extracts of garlic (Ali et al., 1993).

D.4. Garlic and Carcinogenesis

Cancer mortality has increased remarkably all over the world during the last decades. Thus, many therapeutic anti-carcinogenic agents have been developed. It is notable that anti-cancer compounds are also found in the diet; and garlic is one dietary source of anti-carcinogens.

Many epidemiological and laboratory studies have demonstrated that garlic has anti-carcinogenic activity (Dorant et al., 1993). In an area of China, increasing consumption of allium vegetables including garlic, sinificantly reduced gastric cancer development (You et al., 1989). Ajoene, a natural sulfur-containing compound in garlic, was tested for cytostatic activity towards primary cultures, a permanent cell line, and a tumorgenic cell line. Ajoene was most cytostatic to the tumor cells and least cytostatic to the primary cells. This suggested that tumor cells are more senstive to ajoene than non-tumor cells (Scharfenberg et al., 1990). Diallyl disulfide, an organosulfur metabolite found in garlic, suppressed dimethylhydrazine (DMH)-induced colon cancer and nitrosa methylbenzylamine (NMBA)-induced oesophageal cancer in animals (Sumiyoshi and Wargovich, 1989).

Basically, carcinogenesis involves initiation, promotion, and progression stages. In order to prevent or inhibit cancer, dietary anti-carcinogens should affect one or more of the three broad stages of carcinogenesis. First, carcinogenicity can be reduced by the biotransforming enzymes such as cytochrome p450 and mixed function oxidases that can be induced by anti-carcinogens. Second, initiation may be blocked by preventing covalent binding of cancer causing substances to the DNA. Furthermore, tumor promotion may be inhibited by many compounds including retinol, tocopherol, and organosulfates. Garlic and onion, rich sources of organosulfates, are considered as inhibitors of tumor promotion (Davis, 1989).

In a liver tumor model initiated by 1,2-dimethylhydrazine (DMH) and promoted by orotic acid, diallyl-dilsulfide (DAS), isolated from garlic, inhibited the promotion process (Hayes et al., 1987). In a mouse skin tumor model, which was initiated by 7,12-dimethylbenz[a]anthracene (DMBA) and promoted by phorbol-myristate-acetate (PMA), onion and garlic extracts elicited a dose-dependent inhibition on PMA promotion (Belman, 1983). PMA stimulates prostaglandin synthesis also (Bresnick et al., 1979; Ashendel et al., 1979).

Lau et al. (1993) suggested several possible cancer protective mechanism for compounds in garlic. The host immune response may be modulated by garlic to act against tumor growth. In addition, compounds in garlic could directly inhibit tumor cell metabolism or inhibit the initiation and/or promotion process of carcinogenesis. Since prostaglandins and related eicosanoids play an important role in the tumor promotion stage of carcinogenesis, the anti-carcinogenic activity of organosulfur compounds may be attributed to inhibition of tumor promotion by inhibiting prostaglandin biosynthesis.

E. JUSTIFICATION

Several studies have indicated that the organosulfur compounds in garlic inhibit biosynthesis of PGE. PGE is a chemoattractant to macrophages which release H_2O_2 that in turn causes oxidative damage and cancer promotion. Macrophages also secrete growth factors and proteases, two critical biomolecules required during tumor promotion and progression. Thus, overproduction of PGE may be a critical step in the overall promotion and progression stages of carcinogenesis.

F. OBJECTIVE

The objective of this study was to develope a method to determine the prostaglandin E metabolite in urine and to determine if a garlic extract would suppress prostaglandin E production in humans.

G. HYPOTHESIS

The main hypothesis of this study is that an aqueous-ethanol garlic extract reduces PGE biosynthesis and urinary PGE-M excretion in women.

H. NULL HYPOTHESIS

The null hypothesis to be tested in this study is that consumption of an aqueous-ethanol garlic extract by women will not cause a difference in PGE biosynthesis and urinary PGE-M excretion.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN. The overall experimental design was specified by the National Cancer Institute (NCI). Eight women consumed garlic for three months in addition to their regular daily diet. The efficacy of garlic in suppressing prostaglandin E synthesis was assessed by measuring the prostaglandin E metabolite (PGE-M) excretion before and after three months of garlic supplementation. This study was approved by the Michigan State University Committee on Research Involving Human Subjects (IRB# 90-480).

SUBJECTS. Eight female subjects, 21 to 41 years of age, were selected from among the staff at the Life Sciences building at Michigan State University. All were healthy with no evidence of any disease based on history, physical examination, cell blood count, urinalysis, and biochemical profile. All subjects signed an informed consent form prior to participation in the study.

GARLIC SUPPLEMENTATION. The garlic supplement (Kyolic™ concentrate) used in this study was an aqueous-ethanol garlic extract provided by the NCI. The organosulfur composition of the supplement was determined by Dr. L.D. Lawson at the Plant Bioactives Research Institute (Springville, Utah). The analysis was not available until after the feeding study was completed. Ten ml of the Kyolic™ concentrate was taken at breakfast every day for three months in orange or vegetable (V8) juice (Campbell Co., Camden, NJ).

URINE SAMPLES. Twenty-four hour urinary excretion was collected six times as shown in Table 2. Total volume of each collection was measured, and then stored at -20°C. After samples were thawed and thoroughly mixed, a 20 ml aliquot was acidified to pH 3.5 for PGE-M analysis. Only the zero time and the three month specimens were analyzed due to the difficulty in measuring PGE-M.

Additional twenty-four hour urine collections were made for three different days from a 28 yr female to establish methodology. Also, urine samples were collected from one individual (male, 49 years of age) before and after taking aspirin. Aspirin was used as a "positive control treatment". This individual did not take any vitamin supplements or medicine for at least three days prior to the urine collection. A baseline (no aspirin) 24 hr urine collection and two 24 hr urine collections during aspirin treatment were made. Urine collections during aspirin treatment started 6 hours after the first aspirin treatment (650 mg, 4 times/day). Each 24 hr urine sample was collected in silanized glass bottles, and kept at 4°C during the collection period.

REAGENTS AND MATERIALS. The internal standard, seven deuterium labeled PGE-M ([$^{2}H_{7}$]-PGE-M), was chemically synthesized as described by Meese (1992). A 50 ng/µl stock

Table 2. Garlic supplemention and urine collection protocol.

	STUDY PERIOD					
Urine collection	-1(wk)	0(mo)	1(mo)	2(mo)	3(mo)	4(mo)
Supplement		Start----------------------End				

solution was prepared in absolute ethanol. All organic solvents were either chromatography grade or redistilled before use. Glassware silanizing reagent, dimethyldichlorosilane (DMCS), was from Aldrich (Milwaukee, WI). Fifty percent formic acid was prepared from 80% concentrated formic acid with deionized water. The Pierce MOX®, and BSTFA - 1%TMCS reagents were used for methoximine and trimethylsilate derivatiation respectively. C_{18} Sep-Pak (500 mg) and silica Sep-Pak (500 mg) solid phase extraction columns were purchased from Waters Associates (Milford, MA). One ml cone shaped vials with teflon lined caps were purchased from Kimble (Vineland, NJ).

EQUIPMENT. Gas chromatography- mass spectrometry (GC-MS) was done with a Hewlett Packard 5890 Gas Chromatography and a 5970B Mass Selective Detector (MSD) (Figure 6). A DB™ - 5ms capillary column (15 M x 0.25 mm I.D, 0.25 µM film thickness) was used to separate the analytes. The GC oven was temperature programmed from 190°C to 250°C at 5°C/min, from 250°C to 310°C at 30°C/min, and held at 310°C for 1 min.

METHODS

GLASSWARE PREPARATION. All analytical operations were done in silanized glassware. Glassware was soaked with 10% Dimethyldichlorosilane (DMCS) for 5-10 min. Excess DMCS was removed by rinsing with toluene, drying, soaking in methanol for 5-10 min, rinsing with excess fresh methanol, rinsing

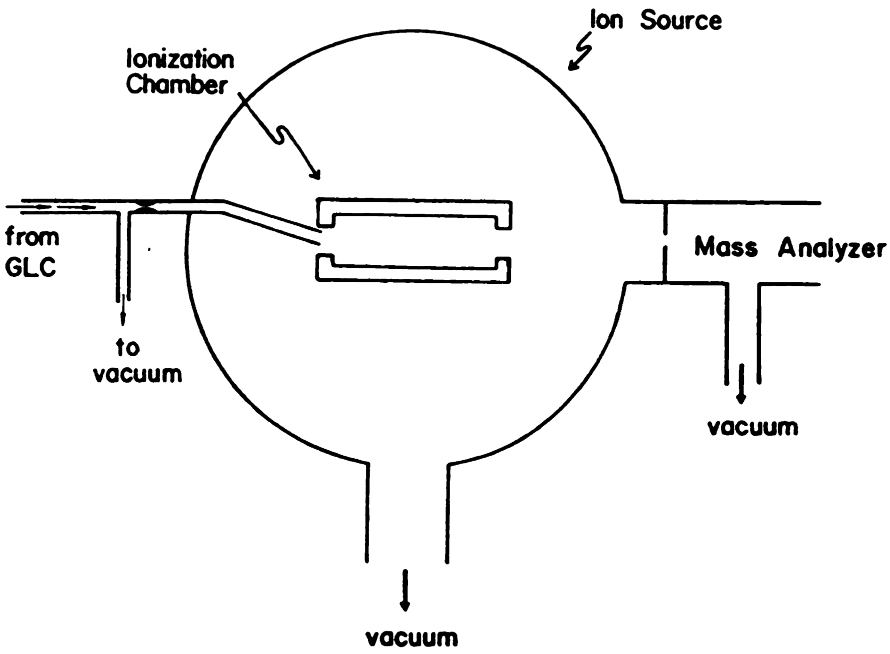


Figure 6. Schematic diagram of a Gas chromatography-Mass spectromtry.

(Adapted from Watson, 1985)

with toluene and drying before use.

PGE-M Extraction from Urine. The overall procedure for PGE-M extraction from urine was as described by Ferretti, et al., (1983) with slight modification (Figure 7). Twenty ml of urine was acidified to pH 3.5 by adding 50% formic acid. Deuterium labeled PGE-M internal standard (200 ng) was added to the acidified urine and mixed with 20 ml of conditioned XAD-2 resin in a 50 ml silanized glass beaker at 4^{o}C for 1 hr. After filtration with No.50 filter paper under suction, the resin was rinsed with 500 ml deionized water, and then 100 ml of petroleum ether. The organic materials of interest were eluted with a total of 120 ml absolute ethanol in sequential 30 ml rinses. The extract was rotoevaporated at room temperature, then the residue was transferred in a small amount of ethanol into a 20 ml silanized glass tube and the ethanol was evaporated under N_2 gas at 38^{o}C. The ethanolic extract residue was methylated as described below. The methylated residue was dissolved in 0.5 ml methanol, 9.5 ml of H_2O/MeOH (85:10, v/v) was added and the mixture was passed through a conditioned octadecylsilyl (ODS) silica (Sep-Pak C_{18} cartridge). The C_{18} cartridge was rinsed sequentially with 20 ml deionized water, 10 ml of petroleum ether, and 5 ml of methyl-formate/petroleum ether (5:95, v/v). PGE-M, still bound to the C_{18} column, was eluted with 5 ml of methyl-formate/petroleum ether (25:75, v/v) and collected in a silanized screw capped tube. After solvent evaporation under nitrogen, the residue was dissolved in 100 µl of ethyl

20 ml Urine + 200 ng of [$^2H_7$]-PGE-M
↓
mix with XAD-2 resin
↓
elute PGE-M with 100% Ethanol
↓
Methylate
↓
clean up with C_{18} Sep-Pak
↓
clean up with Silica Sep-Pak
↓
Methoximate (MO)
↓
Trimethylsilate (TMS)
↓
GC-MS
Selected Ion Monitoring (SIM)

Figure 7. Scheme for the analysis of urinary PGE-M.

acetate/2,2,4-trimethyl pentane (2:1, v/v), and chromatographed over a 3 ml silica gel column. The PGE-M methyl ester was collected in the third to eighth ml of eluent (ethyl acetate/2,2,4-trimethyl pentane (2:1, v/v)). After evaporation of solvent under nitrogen, methoximine and trimethylsilyl ethers were prepared as described below.

DERIVATIVE FORMATION

Methylester formation (ME). Methanol (0.5 ml) and 2 ml of ethereal diazomethane solution were added to the dried ethanol eluate and vortexed thoroughly. After reacting for 10-15 min at room temperature, the solvent was evaporated under a nitrogen stream.

Methoximation (MO). Methoximines were prepared by adding 125 µl of MOX® reagent to the dried residue eluted from the silica gel Sep-Pak, vortexing thoroughly and heating for 3 hours at 60°C. The pyridine was evaporated, 0.5 ml of H_2O was added, and the methoximated derivatives were extracted three times with 0.5 ml benzene. The benzene extracts were combined for further derivatization.

Trimethylsilation (TMS). The dried residue from the benzene extract of methoximated compounds was treated with 100 µl of the BSTFA - 1%TMCS reagent. After incubation at 80°C for 1 hr, the pyridine was evaporated and the residue was redissolved in 10 ul of ethyl acetate. The sample was then ready for GC-MS analysis.

Since the endogenous PGE-M and the $[^2H_7]$-PGE-M were processed through the entire assay together, the efficiencies of isolation, purification, and derivitization and the extent of sample loss should be comparable for both the endogenous PGE-M and the internal standard PGE-M. The $[^2H_7]$-PGE-M was utilized to identify the retention time of the endogenous PGE-M and to estimate the quantity of endogenous PGE-M as described in the section below. The chemical and physical characteristics for the endogenous PGE-M and the $[^2H_7]$-PGE-M standard are identical except for the mass difference of seven that comes from the deuterium atoms in the internal standard.

QUANTITATIVE ANALYSIS OF PGE-M BY GC-MS

PGE-M was quantitated by GC-MS. Two ions, m/z 365 and m/z 372, were determined in the Selected Ion Monitoring (SIM) mode. Endogenous PGE-M was identified at m/z 365 according to the retention time of the internal standard PGE-M on the mass chromatogram. Quantification of endogenous PGE-M was calculated by the equation:

$$\frac{\text{Endogenous PGE-M}}{[^2H_7]\text{-PGE-M}} = \frac{\text{PEAK AREA}_{\text{endogenous PGE-M (m/z365)}}}{\text{PEAK AREA}_{\text{[2H7]-PGE-M (m/z372)}}}$$

Since PGE-M slowly degenerates during storage, correction for decay of PGE-M during urine storage was done as described by Seyberth el al., (1976) . PGE-M has a half life of 318 days; the data were corrected by the equation:

$\ln C = \ln C_o - 0.002180t$ where C = estimated PGE-M concentration, C_o = adjusted PGE-M concentration, and t = days of storage before PGE-M analysis.

STATISTICAL METHODS

GC-MS detector response for PGE-M injection was tested by linear regression analysis. Garlic treatment effect on urinary PGE-M excretion was evaluated by the paired t-test.

RESULTS AND DISCUSSION

In this study, the measurement of urinary PGE-M was done by SIM since SIM can distinguish the [$^2H_7$]-PGE-M from the endogenous PGE-M and because of the high sensitivity and selectivity of SIM GC-MS. Prior to analysis by GC-MS, both the endogenous and internal standard PGE-M had to be extracted, concentrated and partially purified from the complex mixture of biological compounds in urine specimens. Sample clean up was absolutely essential to minimize interference from ions derived from the overwhelming amounts of other urinary compounds relative to PGE-M. In addition, in order to increase thermal stability and volatility and to decrease polarity, PGE-M had to be derivatized for GC analysis. PGE-M, the 11α-hydroxy-9,15,-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid, with its one hydroxyl, two keto, and two carboxyl groups, was derivatized to the trimethylsilyl ether, o-methyloxime, and methyl ester (ME-MO-TMS) derivative which could then be analyzed by GC-MS (Figure 8). Figure 9 shows the TIC chromatogram for the ME-MO-TMS derivative of [^2H]$_7$-PGE-M under the GC conditions that were described in the Materials and Methods section.

The molecular ion (M^+), or the intact ionized molecule of the ME-MO-TMS [^2H]$_7$-PGE-M, was determined at a m/z of 493.

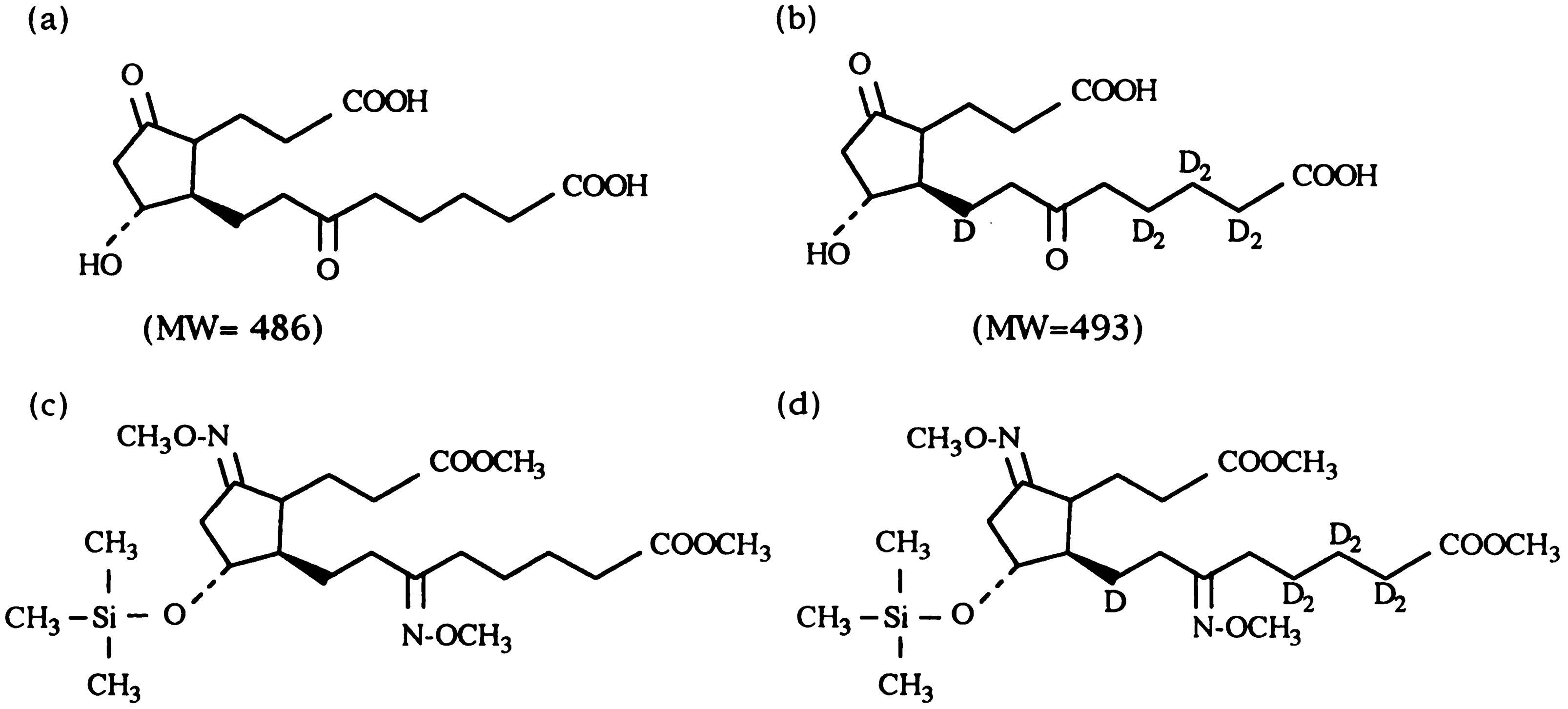


Figure 8. Chemical structures of PGE-M, [^{2}H7]-PGE-M and their MO-MO-TMS derivatives.

(a) PGE-M, the 11α-hydroxy-9,15,-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid.

(b) [^{2}H7]-PGE-M, the [13,17,17,18,18,19,19-D7]-19-carboxy-9,15,-dioxo-11α-hydroxy-2,3,4,5,20-pentanor prostanoic acid.

(c) ME-MO-TMS PGE-M , the bis-methyloxime-dimethyl ester≥-trimethylsilyl ether derivative of PGE-M.

(d) ME-MO-TMS [^{2}H7]-PGE-M, the bis-methyloxime-dimethyl ester-trimethylsilyl ether derivative of [^{2}H7]-PGE-M.

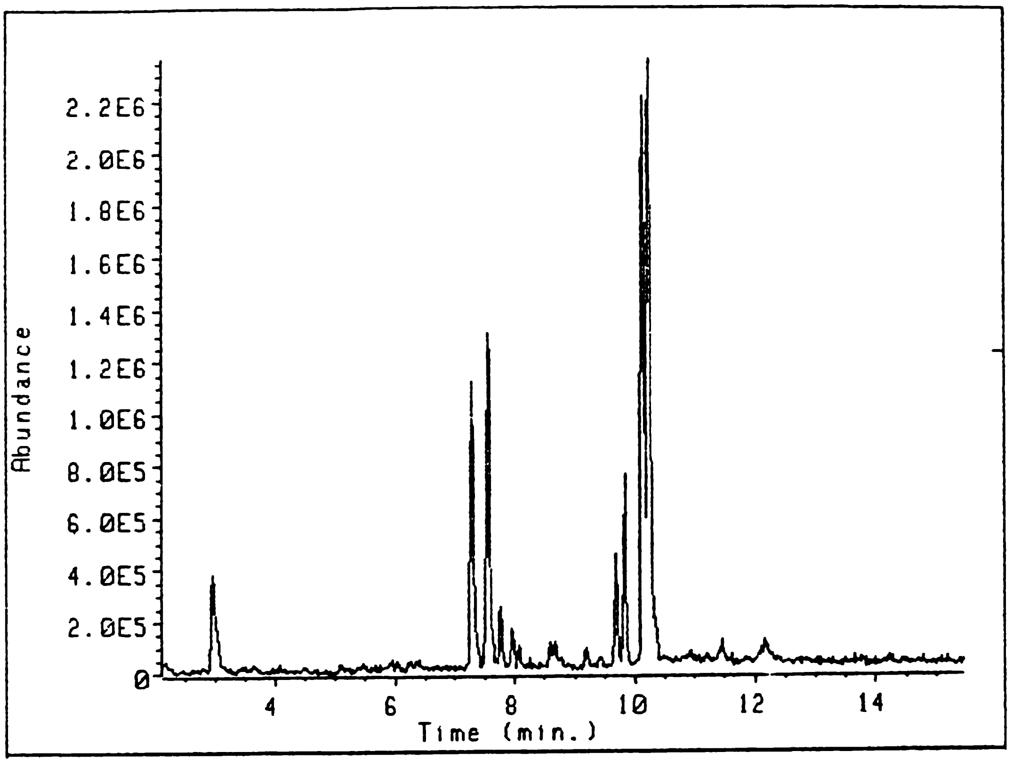


Figure 9. Total ion current (TIC) chromatogram of ME-MO-TMS $[^{2}H_{7}]$-PGE-M.

The base peak, the most intense peak in the mass spectrum, of the ME-MO-TMS [^{2}H]$_7$-PGE-M was determined at m/z 372 which represents the [M$^+$ minus (OCH$_3$) minus (CH$_3$)$_3$ minus SiOH)] ion ([M$^+$-OCH$_3$-(CH$_3$)$_3$-SiOH]). Scanning the mass spectrum of each peak in Figure 9 showed that the base peak was the predominant ion detected for peaks with retention times of 10.241 min (Figure 10a), 10.132 min (Figure 10b), 9.838 min (Figure 11a), and 9.701 min (Figure 11b). The peaks at 7.544 min (Figure 12a) and 7.289 min (Figure 12b) had a different pattern of mass to charge ions. These peaks were found in "reagent blanks" and most likely resulted from the derivatization reagents.

The highest mass shown on the mass spectrum in Figure 10 and Figure 11 was m/z 462 instead of the molecular ion (M$^+$) of m/z 493. Frequently, the M$^+$ is not found in a mass spectrum because all of the analyte is fragmented within the mass chamber and none of the parent compound with a mass of m/z 493 remains. The $\cdot$OCH$_3$ radical was released from the oximine moiety of the M$^+$ and this [M-31]$^+$ fragment was then detected at m/z 462 (Figure 13). However, the predominant fragment was [M$^+$ - $\cdot$OCH$_3$ - $\cdot$(CH$_3$)$_3$ SiOH], ([M-121]$^+$), which was detected at m/z 372. Figure 14 shows the mass chromatogram when the GC-MS was operated in the SIM mode and the m/z 372 was monitored. Figure 14 is consistent with the TIC for the [^{2}H$_7$]-PGE-M derivative for retention times 9.6-10.5 min as shown in Figure 9. Four peaks were produced by the standard since each keto group produced a syn- and an

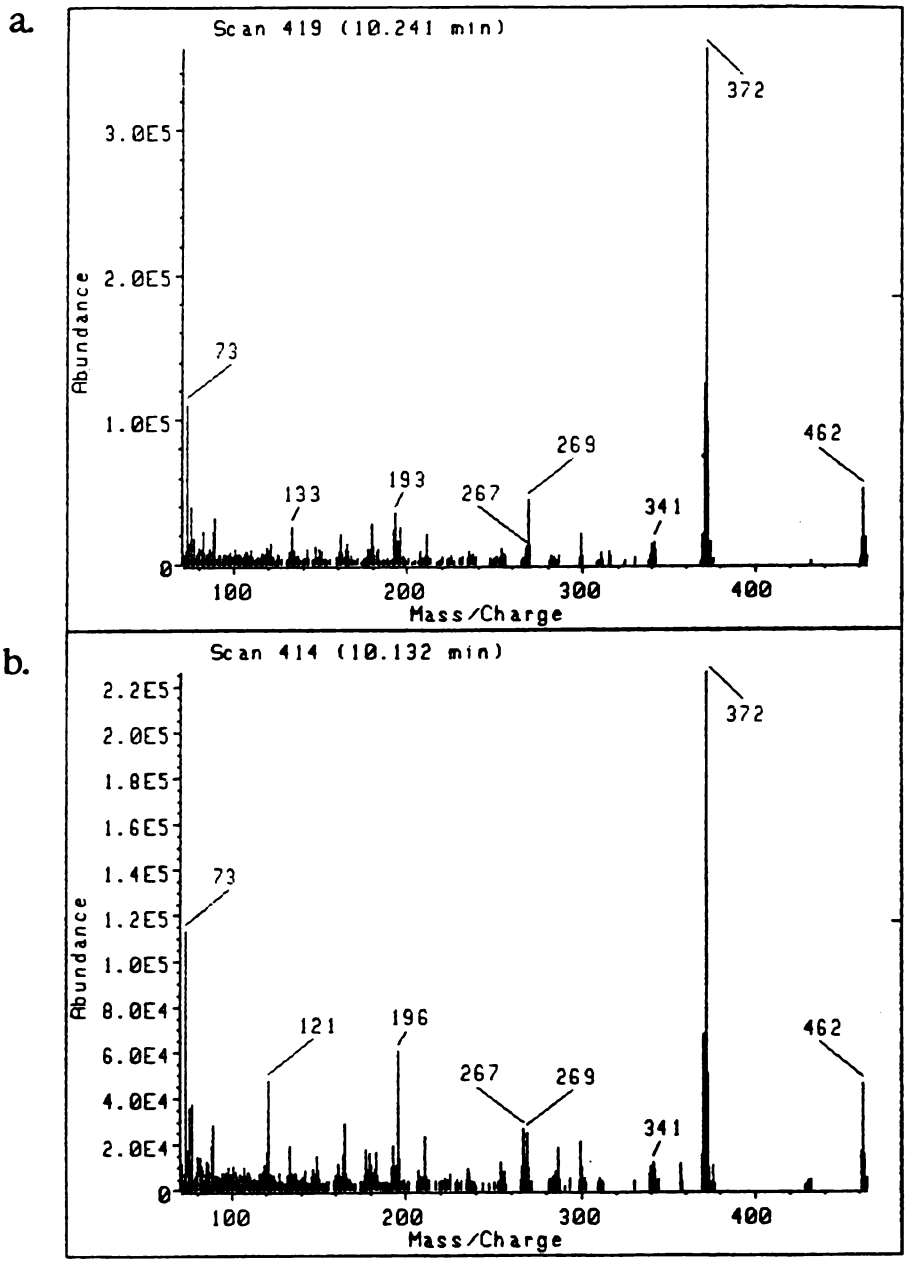


Figure 10. Scans of the mass spectrum of the most intense peaks in the TIC chromatogram of ME-MO-TMS [$^2H_7$]-PGE-M at the retention time of 10. 241min (a) and 10.132 min (b). The m/z 372 ion is the base peak shown in both mass spectrums.

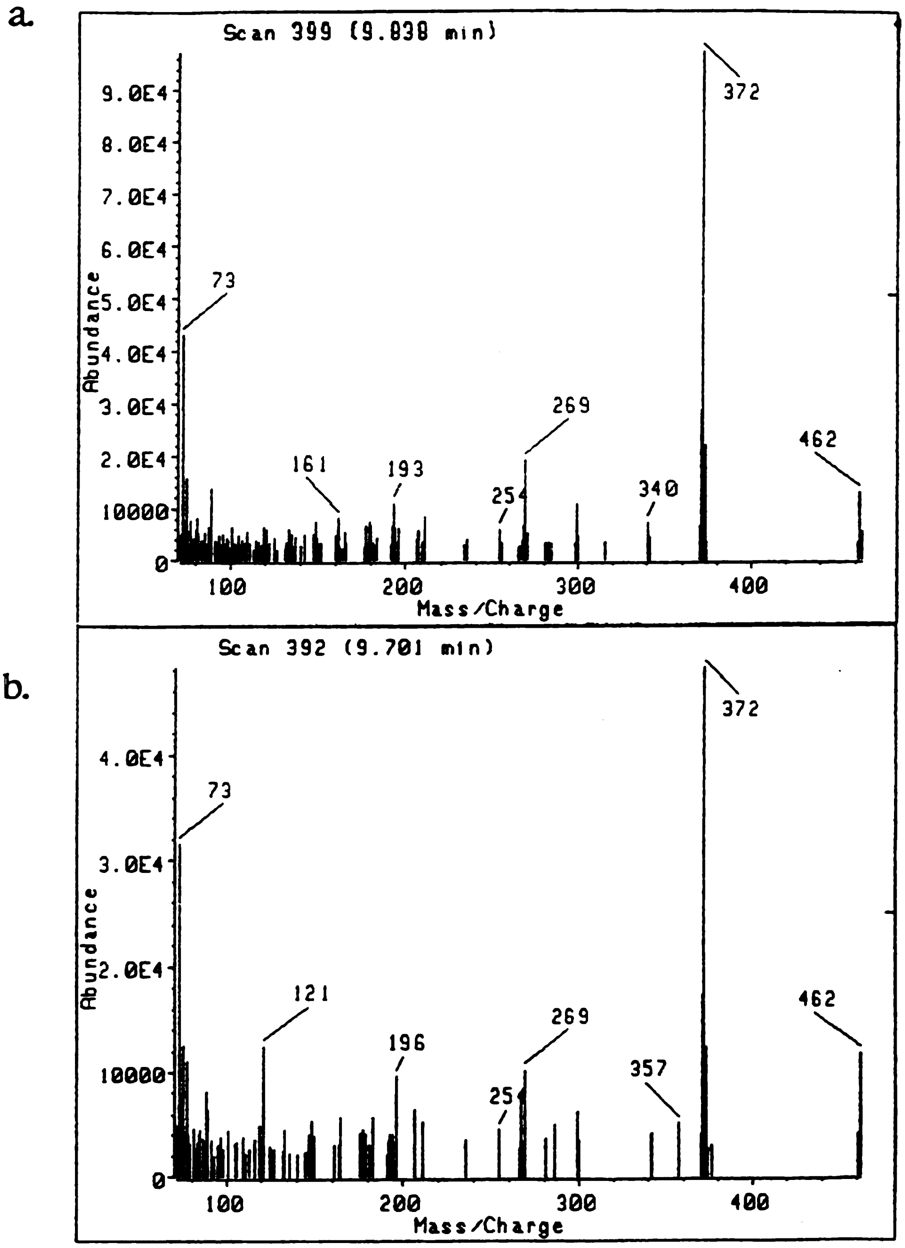


Figure 11. Scans of the mass spectrum of the most intense peaks in the TIC chromatogram of ME-MO-TMS [$^2H_7$]-PGE-M at the retention time of 9.838min (a) and 9.701 min (b). The m/z 372 ion is the base peak shown in both mass spectrums.

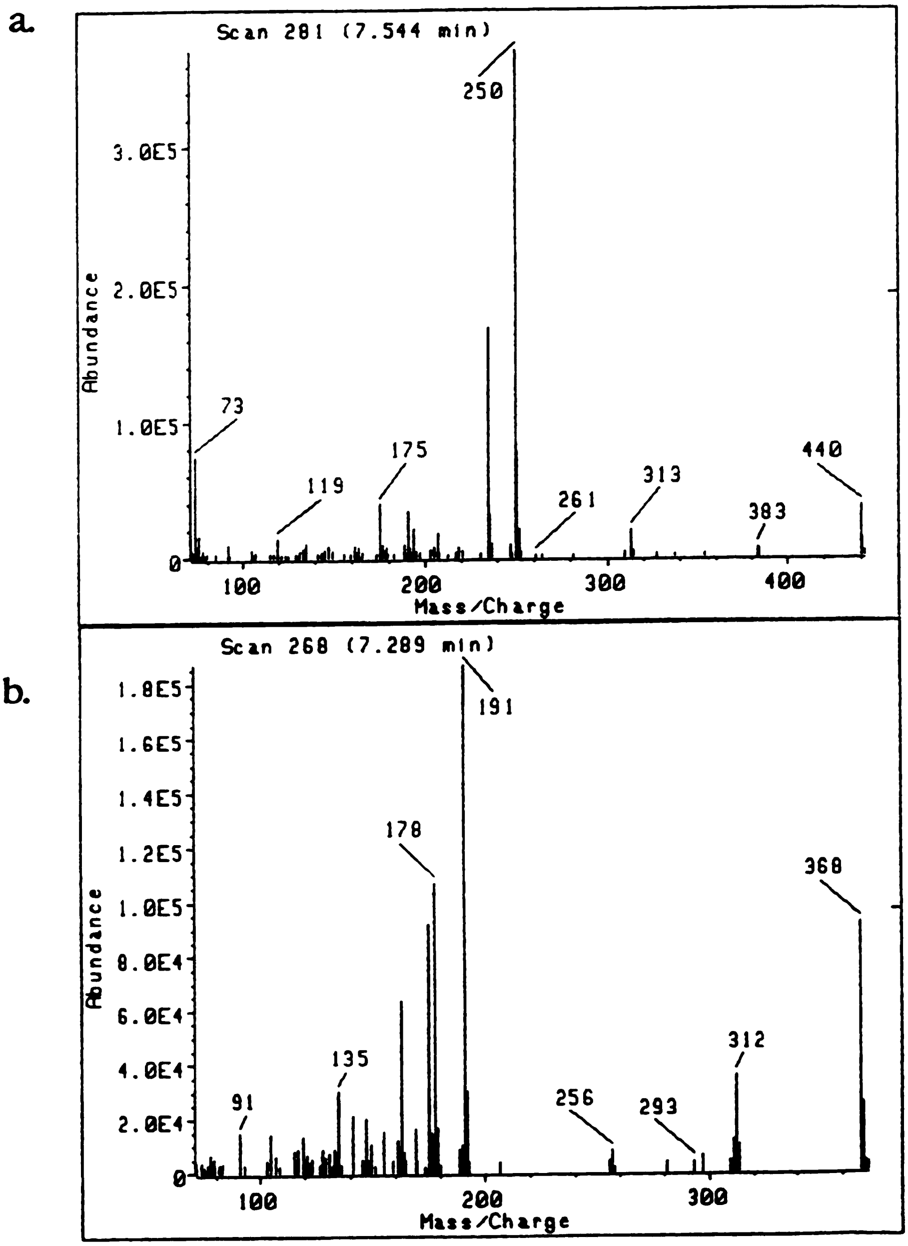


Figure 12. Scans the mass spectrum of the intense peaks in the TIC chromatogram of ME-MO-TMS [$^2H_7$]-PGE-M at the retention time of 7.544min (a) and 7.289 min (b). Compared with the previous mass spectrums in Figure 10 and Figure 11, different spectrum patterns are produced, and the m/z 372 ion is not present.

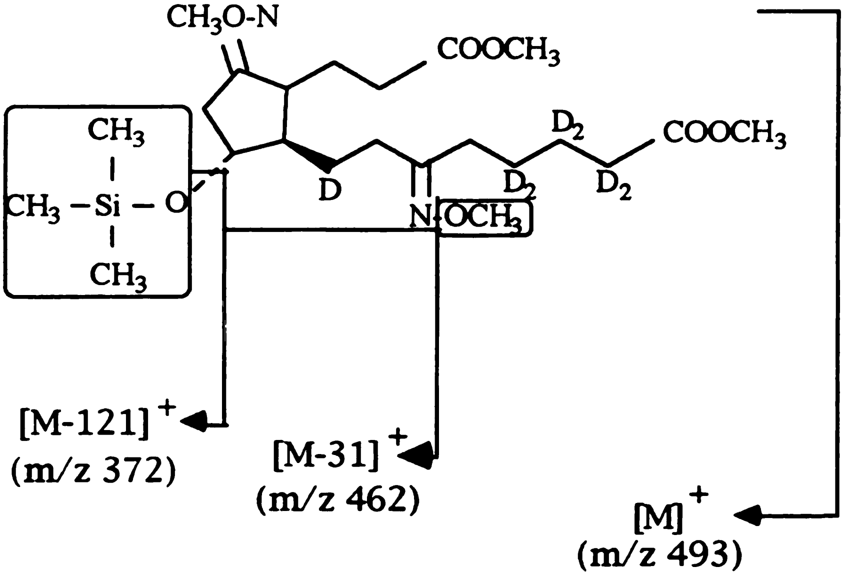


Figure 13. Analyte fragmentation occurrs within the mass chamber with release of ·OCH3 and ·(CH3)3SiOH radicals from the molecular ion (M^+, m/z 493). If only ·OCH3 is released, the $[M-31]^+$ fragment is formed and the m/z 462 ion is produced; if both ·OCH3 and ·(CH3)3 SiOH are released, then the $[M-121]^+$ fragment is formed and the m/z 372 ion is produced.

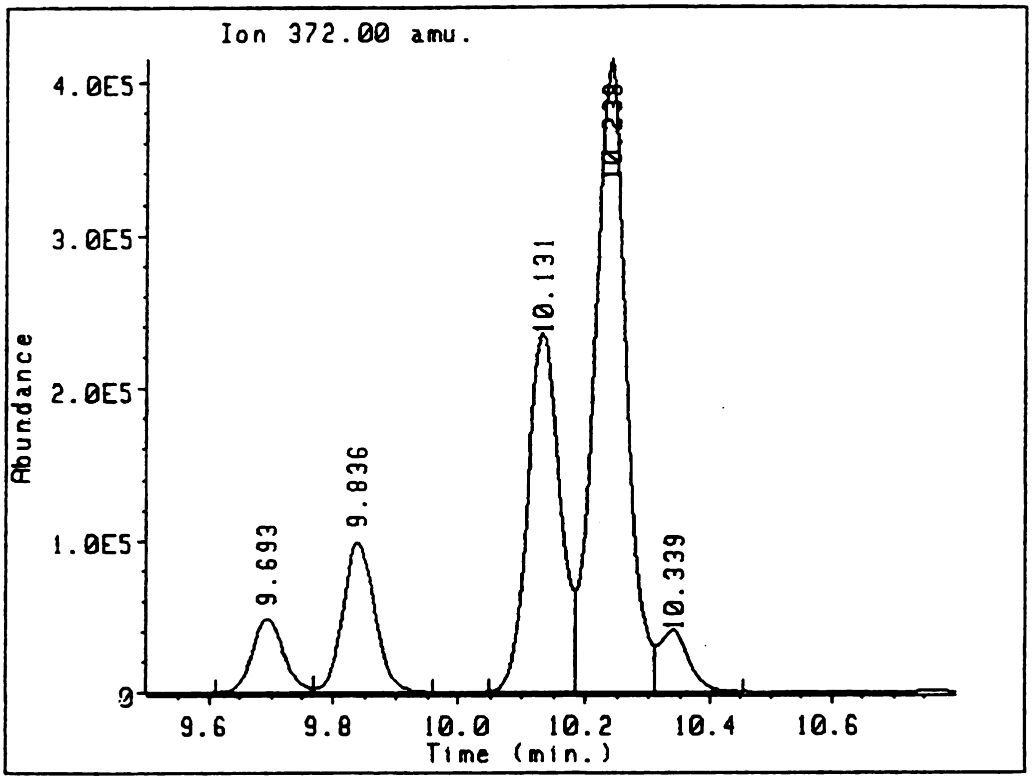


Figure 14 . From the TIC profile of ME-MO-TMS [$^2H_7$]-PGE-M, the m/z 372 ion was monitored to create a new mass chromatogram, which identified the retention time of this standard compound.

anti- methoxime isomer; therefore, four isomers were formed from the two keto groups on PGE-M.

Various amounts of ME-MO-TMS [$^2H_7$]-PGE-M were injected into the GC-MS and the abundance of the m/z 372 ion was recorded. A linear relationship (Y = 161839 + 289758 (r= 0.992)) was observed when 0.1, 0.5, 1.0, 5.0, 10.0, 100.0, and 200.0 ng of PGE-M were injected. The regression equation and the 95% confidence intervals are shown in Figure 15. These data demonstrate that the abundance of the m/z 372 ion was proportional to the amount of PGE-M injected into GC-MS.

Figure 16 shows a typical chromatograph for quantification of endogenous PGE-M. Two hundred ng of [$^2H_7$]-PGE-M was added to 20 ml of urine (from a female, 28 years of age) and the PGE-M was extracted as described in the Materials and Methods section. The abundance of two ions, m/z 365 and m/z 372 which correspond to the [M^+-($\cdot OCH_3$+ $\cdot (CH_3)_3SiOH$)] fragments for endogenous PGE-M and [$^2H_7$]-PGE-M respectively, were monitored in the SIM mode. The four isomers of endogenous PGE-M and of [$^2H_7$]-PGE-M are noted on the chromatogram.

The urinary PGE-M excretion from this subject was estimated as 315.9 ± 33.1 ng/20 ml (mean ± SEM, three 24 hr samples) which was similar to that reported (395 ± 22 (SE) ng/20 ml) by Ferretti, et al.(1983). The 24 hr urinary PGE-M excretion was calculated as 18.2 ± 1.9 μg/24 hr (mean ± SEM, three 24 hr samples).

Aspirin and NSAIDs are potent prostaglandin biosynthesis

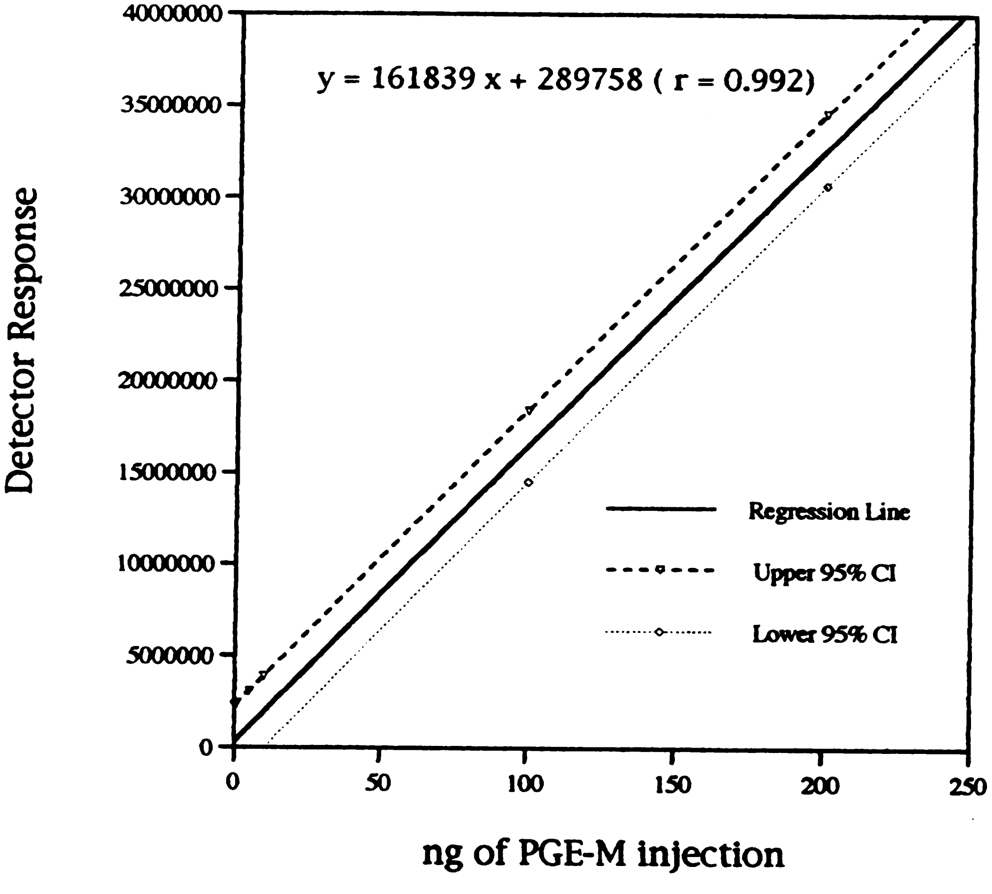


Figure 15. The relationship between amount of $[^2H_7]$-PGE-M injected (0.1 ng - 200 ng) and the GC-MS detector response.

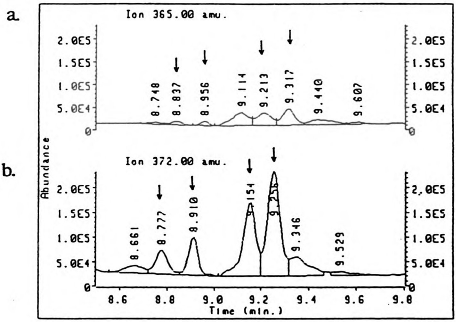


Figure 16. SIM chromatograms of an urine sample. The mass chromatogram of endogenous PGE-M is shown at m/z 365(a) and the [$^2H_7$]-PGE-M internal standard is shown at m/z 372 (b). The endogenous PGE-M peaks (panel a) relative to the internal standard peaks (panel b) are identified by the arrow symbols (↓). The peaks identified by (↓) are isomers.

inhibitors (Vane et al., 1971; Flower, 1974). Therefore, the excretion of PGE-M before and after aspirin (acetyl salicylic acid) treatment was determined in a 49 year old male to serve as a "positive control treatment"; the results are shown in Table 3. Aspirin treatment (4 x 650 mg aspirin/day) reduced urinary PGE-M production to 19% to 47% of baseline value. Seyberth (1976) reported that aspirin treatment (4 x 650 mg aspirin per day caused a 45% to 70% decrease in PGE-M excretion in eight 19-30 yrs males.

The effect of garlic treatment on the PGE-M excretion in eight women is summarized in Table 4. The mean baseline (before treatment) PGE-M value is 42.7 ± 9.5 (mean ± SEM, N=8) and the PGE-M excretion after three month of garlic treatment is 35.9 ± 4.7 (mean ± SEM, N=8). PGE-M excretion during baseline and after garlic treatment were not significantly different as determined by the paired t test (P = 0.29). The aqueous-ethanol garlic extract did not decrease the PGE-M excretion as expected. There may be several reasons why the garlic extract did not inhibit prostaglandin synthesis. First, the organosulfur compounds that are in the aqueous-ethanol garlic extract (Table 5) may not be active inhibitors of prostaglandin biosynthesis. Allicin and ajoene are the most potent inhibitors of prostaglandin synthesis (Block, 1985). However, the concentration of these two compounds in the Kyolic™ concentrate are very low (Table 5). The amounts of S-Allyl-mercapto-cysteine, S-trans-1-propenyl-cysteine and

Table 3. Twenty-four hour urinary PGE-M excretion before and after aspirin treatment (4 x 650 mg/day).

	PGE-M (μg/ 24 hr)[a]	Percentage (%)
Baseline	44.1 ± 2.0	100 %
Aspirin Day 1	8.2 ± 1.2	19 %
Aspirin Day 2	20.7 ± 1.6	47 %

[a] Values are mean ± CV.

Table 4 . Twenty-four hour urinary PGE-M excretion (μg/ 24 hr) for eight women before and after three months consumption of an aqueous-ethenol garlic extract.

	PGE-M (μg/24 hr)[a]	
Subject	Baseline	Garlic Treatment
No. 1	35.8	42.8
No. 2	38.5	30.2
No. 3	72.4	35.0
No. 4	12.1	56.6
No. 5	89.7	24.5
No. 6	21.6	46.9
No. 7	50.8	37.0
No. 8	20.3	13.9
mean ± SEM	42.7 ± 9.5	35.9 ± 4.7

[a] Analyzed in duplicate.

Table 5. Organosulfur compounds in Kyolic™ concentrate and garlic. [a]

Compound	Kyolic™ [b] (micrograms/gram)	Crushed [c] Garlic (fresh wt.) (micrograms/gram)	Fresh dried [d] Garlic (dry wt.) (micrograms/gram)
Alliin (allyl-cysteine-sulfoxide)	610	400	27,400
Allicin	< 2	3900	< 2
γ-Glutamyl-S-allylcysteine	200	4900	8300
S-Methyl-cysteine	270	40	110
S-Allyl mercaptocysteine	185	< 20	< 20
Methionine	95	23	45
Ajoene	< 1	< 1	< 5
γ-Glutamyl-S-trans-1-propenylcysteine	92	4600	8600
S-Allylcysteine	1165	< 20	1080
S-Trans-1-propenylcysteine	530	< 20	310

[a] Analysis was done by Dr. L.D. Lawson (Bioactives Research Institute, Springville, Utah).

[b] The garlic supplement used in this study.

[c] Garlic cloves (68% moisture content) homogenized in water.

[d] Powder of typical store-purchased garlic.

* Allyl sulfide, Diallyl sulfide, Diallyl disulfide, Diallyl trisulfide, methyl allylsulfide, methyl allyl disulfide and methyl allyl trisulfide were present in minimal amounts (less than 10 μg/ml concentrate).

S-Allylcysteine are high in the Kyolic™ concentrate. However, it is not known if they inhibit PG synthesis.

In fresh garlic, a major organosulfur compound is γ-Glutamyl-S-allylcysteine (GSAC). During storage, GSAC is proteolytically hydrolyzed to alliin (Figure 5). Neither GSAC nor alliin is active until chemically transformed to allicin as described previously. It is assumed that GSAC and alliin can be converted to allicin during digestion. So, in the case of fresh and freeze dried garlic, there are large amounts of allicin and precursors (fresh garlic) or allicin precursors (freeze dried). In the Kyolic™ concentrate used in this study, allicin and its precursors are minimal compared to the fresh and freeze dried garlic. Therefore, the effectiveness of Kyolic™ concentrate to inhibit PG synthesis could be very limited because of insufficient inhibitors.

Moreover, during three months of garlic treatment, there may be compensation and up regulation of prostaglandin biosynthesis in response to continued consumption of PG inhibitors. Thus, a small inhibition of PG synthesis by long term treatment with garlic extract may not be detected.

The Kyolic™ concentrate was the choice of the NCI, since it contains S-allylcysteine, trace amounts of diallyl disulfide, and diallyl trisulfide - chemicals that inhibit tumorgenesis (Dorant, 1993). However, those allyl-containing compounds may not be associated with inhibition of PG synthesis.

SUMMARY AND CONCLUSIONS

Garlic (*Allium sativum*) contains organosulfur compounds that inhibit the biosynthesis of prostaglandin E (PGE). PGEs are associated with inflammatory response and the tumor promotion stage of carcinogenesis. Therefore, this study was conducted to determine if an aqueous-ethanol extract of garlic (Kyolic™ concentrate) could inhibit prostaglandin E metabolism.

Eight females (21 to 41 years of age) consumed 10 ml of Kyolic™ concentrate for three months. Twenty-four hour urine collections were made before and after three months of garlic consumption to measure the excretion of the 11α-hydroxy-9,15,-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid (PGE-M) since PGE-M reflects whole body turn over of prostaglandin E_1 and E_2. There was no difference in PGE-M excretion before (42.7 ± 9.5 μg/24 hr)(mean ± SEM, N= 8) and after three months of garlic consumption (35.9 ± 4.7 μg/24 hr)(mean ± SEM, N=8) ($P = 0.29$). These data show that consuming 10 ml of Kyolic™ concentrate daily for three months did not result in a significant decrease in PGE-M excretion in women.

The aged aqueous-ethanol garlic extract (Kyolic™) has relatively low concentrations of prostaglandin synthesis inhibitors (allicin and its precursors) compared to fresh or freeze-dried garlic. The low intake of allicin and precursors is the most likely explanation for the lack of treatment effect in this study.

RECOMMENDATIONS FOR FUTURE RESEARCH

For future research, if the objective is to inhibit PG synthesis, a product rich in allicin and/or ajoene should be selected.

Another consideration should be that, the concentration of urinary PGE-M in a normal individual is extremely low -- in the ng per ml level. Therefore, inhibition of PGE metabolism in normal subjects will be very difficult to detect. Thus, for future studies, the subjects could be carefully selected to have inflammatory disorders, e.g. allergy, asthma, rheumatoid arthritis or cystic fibrosis - patients with these conditions that have elevated prostaglandins production. The effect of garlic treatment on PGE-M excretion could then be examined more efficiently and the results applied to pharmaceutical situations.

In vitro studies would be valuable additions to human studies. For example, several immunocytes, such as macrophages and neutrophils, synthesize significant amounts of prostaglandins. These cells can be isolated from humans or animals and treated with garlic extracts. The production of PGs with or without garlic treatment could be determined. Similar assays could be conducted with the cell lines that synthesize elevated levels of PGs. In addition, cytotoxic effects from various doses of garlic extracts could be also examined.

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